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5 **lovE Variant Regulator Molecules**
(Atty Docket No. 109272.150; Client Docket No. MIC005US)

10 **BACKGROUND OF THE INVENTION**

Field of the Invention

 The invention relates to the fields of microbiology
and molecular biology. In particular, the invention
relates to the field of mycology and the production of
15 secondary metabolites from fungi.

Summary of the Related Art

 Secondary metabolites are a major source of
commercially useful products such as food additives,
20 vitamins, and medicines for the treatment of a wide
variety of infections and diseases. By way of example,
in 1997 the statin drugs lovastatin, simvastatin, and
pravastatin, fungal secondary metabolites used in the
treatment of hypercholesteremia, together had US sales of
25 US\$7.53 billion (Sutherland et al., *Current Opinion In*
Drug Discovery & Development 4:229-236 (2001)). The cost
and availability of these plant, bacterial and fungal
metabolites are frequently determined by limitations
imposed on production and purification of these compounds
30 from culture. This problem is frequently exacerbated by
the fact that these products are generally produced
during the stationary phase of bacterial and fungal
growth.

 A wide variety of methods have been utilized to
35 increase the amount of secondary metabolite produced in
culture. Studies have demonstrated the importance of
carefully designing the medium in which a fungus is grown
to maximize the amount of a secondary metabolite produced
(see, e.g., Hajjaj H, et al., *Appl. Environ. Microbiol.*
40 67:2596-602 (2001); Lesova, K., et al., *J. Basic*
Microbiol. 40:369-75 (2000)). In addition, the method of

5 culture or fermentation also impacts directly on the amount of secondary metabolite produced. For example, see Robinson, T., et al. (*Appl. Microbiol. Biotechnol.* 55:284-289 (2001)), which demonstrates the advantages of solid state (substrate) fermentation.

10 In addition to the manipulation of culture and media conditions, genetic approaches have been taken to increase secondary metabolite production. For example, the production of penicillin is limited by the activity of two enzymes, encoded by the *ipnA* and *acvA* genes, both
15 of which are regulated by the *pacC* protein, a zinc-finger transcription factor. Naturally occurring mutant alleles of the *pacC* locus are known to possess more transcription-activating activity than the cognate, wild-type allele (see, e.g., Tilburn et al. *EMBO J.* 14(4):779-
20 790 (1995)). Thus, one genetic approach to increasing secondary metabolite production is to identify and isolate naturally occurring mutant alleles, the expression of which leads to increased secondary metabolite production.

25 Although many regulators of secondary metabolite production in many organisms are known, not all of the organisms that produce secondary metabolites are amenable to genetic or molecular genetic manipulation. Thus, these systems are not generally useful as a source for
30 the isolation of naturally occurring mutant alleles and are even less useful for the deliberate manipulation of secondary metabolite regulator protein structure with the aim of creating improved regulators of secondary metabolite production.

35 It would be advantageous to have improved regulators of the biosynthetic enzymes responsible for secondary metabolite production. For example, recent studies suggest increasing usage of statin drugs, e.g., see Waters D.D., *Am. J. Cardiol.* 88:10F-5F (2001)). Thus,

- 5 demand for statin drugs is likely to increase substantially. In order to meet the demand for these and other secondary metabolites, new and improved methods for the production of secondary metabolites must be identified.

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BRIEF SUMMARY OF THE INVENTION

The invention provides improved secondary metabolite regulator proteins that enable increased production of secondary metabolites. The invention also provides methods to make these improved regulator proteins.

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In a first aspect, the invention provides a variant regulator protein of secondary metabolite production with increased activity than that of the cognate, wild-type protein. In certain embodiments of this aspect of the invention, the regulator protein is a fungal regulator protein.

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In an embodiment of the first aspect, the invention provides an improved regulator protein comprising an amino acid sequence coding for a variant lovE protein having at least one specific mutation that gives rise to greater transcription-activating properties of the regulator protein and/or induction of secondary metabolite synthesis.

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By way of non-limiting example, certain preferred regulator proteins of this aspect of the invention include at least one of the following mutations: (1) a Group 6 amino acid residue mutated to a Group 2 amino acid residue at position 31, in one embodiment the mutation represented by F31L; (2) a Group 3 amino acid residue mutated to a Group 5 amino acid residue at position 41, in one embodiment the mutation represented by Q41K or Q41R; (3) a Group 4 amino acid residue mutated to a Group 2 amino acid residue at position 52, in one embodiment the mutation represented by T52I; (4) a Group 4 amino acid residue mutated to a Group 3 amino acid residue at position 52, in one embodiment the mutation represented by T52N; (5) a Group 4 amino acid residue mutated to a Group 5 amino acid residue at position 73, in one embodiment the mutation represented by C73R; (6) a Group 1 amino acid residue mutated to a Group 4 amino

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5 acid residue at position 101, in one embodiment the
mutation represented by P101S; (7) a Group 1 amino acid
residue mutated to a Group 3 amino acid residue at
position 101, in one embodiment the mutation represented
by P101Q; (8) a valine amino acid residue mutated to
10 another Group 2 amino acid residue at position 111, in
one embodiment the mutation represented by V111I; (9) a
Group 4 amino acid residue mutated to a Group 2 amino
acid residue at position 133, in one embodiment the
mutation represented by S133L; (10) a Group 3 amino acid
15 residue mutated to a Group 2 amino acid residue at
position 141, in one embodiment the mutation represented
by E141V; (11) a Group 3 amino acid residue mutated to a
Group 5 amino acid residue at position 141, in one
embodiment the mutation represented by E141K; (12) a
20 Group 4 amino acid residue mutated to Group 6 amino acid
residue at position 153, in one embodiment the mutation
represented by C153Y; (13) a Group 4 amino acid residue
mutated to a Group 5 amino acid residue at position 153,
in one embodiment the mutation represented by C153R; (14)
25 a Group 4 amino acid residue mutated to a Group 1 amino
acid residue at position 281, in one embodiment the
mutation represented by T281A; (15) a Group 3 amino acid
residue mutated to a Group 2 amino acid residue at
position 367, in one embodiment the mutation represented
30 by N367I; (16) a Group 3 amino acid residue mutated to a
Group 6 amino acid residue at position 367, in one
embodiment the mutation represented by N367Y; (17) a
Group 1 amino acid residue mutated to Group 4 amino acid
residue at position 389, in one embodiment the mutation
35 represented by P389S; and (18) a Group 1 amino acid
residue mutated to a Group 2 amino acid residue at
position 389, in one embodiment the mutation represented
by P389L.

5 In some embodiments of the first aspect, the invention provides regulator proteins with at least two, or at least three, or at least four, or at least five, or at least six, or at least seven, or at least eight, or at least nine, or at least ten, or at least eleven, or at least twelve, or at least thirteen, or at least fourteen, or at least fifteen, or at least sixteen, or at least seventeen, or at least eighteen of the above described specific mutations.

15 In other embodiments of the first aspect, the invention provides an isolated lovE variant regulator protein selected from the group consisting of SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65.

25 In a second aspect, the invention provides a nucleic acid molecule encoding a lovE regulator of the first aspect of the invention. By way of non-limiting example, the invention provides a nucleic acid molecule encoding the lovE variant regulator protein selected from the group consisting of SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, and SEQ ID NO:90.

35 In a third aspect, the invention provides a method of increasing the activity of a protein that regulates secondary metabolite production comprising: (a) selecting a nucleic acid comprising a polynucleotide encoding a protein regulator of secondary metabolite production; (b)

5 mutating the nucleic acid to create a plurality of
nucleic acid molecules encoding variant regulator
proteins of secondary metabolite production; and (c)
selecting a variant regulator protein with more activity
than the cognate, wild-type protein.

10 In various embodiments of the third aspect, the
secondary metabolite is a fungal secondary metabolite.
In certain embodiments of the third aspect, the protein
regulator of secondary metabolite production is a
transcription factor. In certain embodiments of the third
15 aspect, the protein regulator of secondary metabolite
production is a transmembrane transporter, protein that
mediates secretion, kinase, G-protein, cell surface
receptor, GTPase activating protein, guanine nucleotide
exchange factor, phosphatase, protease,
20 phosphodiesterase, bacterial protein toxin, importin,
RNA-binding protein, SCF complex component, adherin, or
protein encoded within a biosynthetic cluster. In certain
other embodiments of the third aspect, the variant
regulator protein is selected to have more activity in a
25 heterologous cell and/or more activity in a homologous
cell than the cognate, wild-type regulator protein. In
certain embodiments, the variant regulator protein is
selected to have more activity in a heterologous cell
and/or more activity in a homologous cell than the
30 cognate, wild-type protein and to cause more secondary
metabolite to be produced in a homologous cell and/or a
heterologous cell when compared to the cognate, wild-type
regulator protein. In a particularly preferred
embodiment, the variant regulator protein is a love
35 variant regulator protein.

In a fourth aspect, the invention provides a method
of increasing production of a secondary metabolite
comprising: (a) selecting a nucleic acid comprising a

5 polynucleotide encoding a protein regulator of secondary
metabolite production; (b) mutating the nucleic acid to
create a plurality of nucleic acid molecules encoding
variant regulator proteins of secondary metabolite
production; (c) selecting a variant regulator protein
10 with more activity than the cognate, wild-type protein;
and (d) expressing the selected variant regulator protein
in a cell, thereby increasing production of the secondary
metabolite in the cell.

In various embodiments of the fourth aspect, the
15 secondary metabolite is a fungal secondary metabolite. In
certain embodiments of the third aspect, the protein
regulator of secondary metabolite production is a
transcription factor. In certain embodiments of the
fourth aspect, the protein regulator of secondary
20 metabolite production is a transmembrane transporter, a
protein that mediates secretion, a kinase, a G-protein, a
cell surface receptor, a GTPase activating protein, a
guanine nucleotide exchange factor, a phosphatase, a
protease, a phosphodiesterase, a bacterial protein toxin,
25 an importin, an RNA-binding protein, an SCF complex
component, an adherin, or a protein encoded within a
biosynthetic cluster. In certain other embodiments of
the fourth aspect, the variant regulator protein is
selected to have more activity in a heterologous cell
30 and/or more activity in a homologous cell. In certain
embodiments, the variant regulator protein is selected to
have more activity in a heterologous cell and/or more
activity in a homologous cell and to cause more secondary
metabolite to be produced in a homologous cell and/or a
35 heterologous cell when compared to the cognate, wild-type
regulator protein. In a particularly preferred

5 embodiment, the variant regulator protein is a love
variant regulator protein.

In a fifth aspect, the invention provides an
isolated variant regulator protein of secondary
metabolite production having increased activity compared
10 to a cognate, wild-type protein, the variant regulator
protein made by the process comprising: (a) selecting a
nucleic acid comprising a polynucleotide encoding a
protein regulator of secondary metabolite production; (b)
mutating the nucleic acid to create a plurality of
15 nucleic acid molecules encoding variant regulator
proteins of secondary metabolite production; (c)
selecting a variant regulator protein with more activity
than the cognate, wild-type protein; and (d) recovering
the selected variant regulator protein.

20 In certain embodiments of the fifth aspect, the
secondary metabolite is a fungal secondary metabolite.
In certain embodiments of the fifth aspect, the protein
regulator of secondary metabolite production is a
transcription factor. In certain embodiments of the fifth
25 aspect, the protein regulator of secondary metabolite
production is a transmembrane transporter, a protein that
mediates secretion, a kinase, a G-protein, a cell surface
receptor, a GTPase activating protein, a guanine
nucleotide exchange factor, a phosphatase, a protease, a
30 phosphodiesterase, a bacterial protein toxin, an
importin, an RNA-binding protein, an SCF complex
component, an adherin, or a protein encoded within a
biosynthetic cluster. In certain embodiments of the
fifth aspect, the variant regulator protein has more
35 activity in a heterologous and/or a homologous cell than
the cognate, wild-type protein. In certain embodiments of
the fourth aspect, the variant regulator protein

5 increases production of a secondary metabolite in a heterologous cell and/or a homologous cell when compared to the cognate, wild-type protein. In a particularly preferred embodiment, the variant regulator protein is a lovE variant regulator protein.

10 In a sixth aspect, the invention provides a fungus having improved lovastatin production made by the process of transforming a fungal cell with a nucleic acid molecule encoding a lovE variant protein of the first aspect of the invention. In an embodiment thereof, the
15 nucleic acid molecule is selected from a nucleic acid molecule of the second aspect of the invention.

In a seventh aspect, the invention provides an improved process for making lovastatin comprising transforming a fungal cell with a nucleic acid molecule
20 encoding a variant of the lovE protein of the first aspect of the invention. In an embodiment thereof, the fungal cell is transformed with a nucleic acid molecule of the second aspect of the invention.

In a eighth aspect, the invention provides a nucleic
25 acid molecule encoding a lovE protein defined by SEQ ID NO:91. In an embodiment thereof, the invention provides an isolated lovE nucleic acid molecule defined by SEQ ID NO:92.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a photographic representation of cells growing on media with and without G418 selection demonstrating *lovFp-HIS3p-Neo* activation in *S. cerevisiae*. Controls include MB968 (vector only), MB2478 (lowly expressed wild-type *lovE*), and MB1644 (highly expressed wild-type *lovE*). All *lovE* variants are expressed in an MB968 vector backbone similar to MB2478.

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Figure 2A is a graphic representation of *lovFp-CYC1p-lacZ* expression in *S. cerevisiae* strains expressing *lovE* variant proteins from the clones *lovE* 1-10.

Figure 2B is a graphic representation of *lovFp-CYC1p-lacZ* expression in *S. cerevisiae* strains expressing *lovE* variant proteins from the clones *lovE* 1-10 from a separate transformation than that of Figure 2A.

Figure 3 is a graphic presentation of *lovFp-CYC1p-lacZ* expression in *S. cerevisiae* strains expressing *lovE* variant proteins from clones *lovE* 16-41.

Figure 4 is a graphic presentation of *lovFp-lacZ* expression in *S. cerevisiae* strains expressing *lovE* variant proteins from clones *lovE* 1-10.

Figure 5 is a graphic presentation of *lovFp-lacZ* expression in *S. cerevisiae* strains expressing *lovE* variant proteins from clones *lovE* 16, 20, 21, 30-34, and 36-41.

Figure 6 is a graphic presentation of lovastatin culture concentration, as measured by enzyme inhibition

5 assay, from broths of *A. terreus* cultures expressing *lovE*
variant proteins 1-10 in.

Figure 7A is a graphic depiction of lovastatin
culture concentration, as measured by HPLC analysis, from
10 broths of *A. terreus* cultures expressing *lovE* variant
proteins 1-10 in MF117.

Figure 7B is a graphic depiction of lovastatin
culture concentration, as measured by HPLC analysis, from
15 broths of *A. terreus* cultures expressing *lovE* variant
proteins 2, 6, 30, 32, 36, 37, 39, and 41 in MF117.

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DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The patents and publications cited herein reflect the level of knowledge in the art and are hereby incorporated by reference in their entirety. Any
10 conflict between any teaching of such references and this specification shall be resolved in favor of the latter.

The invention utilizes techniques and methods common to the fields of molecular biology, genetics and microbiology. Useful laboratory references for these
15 types of methodologies are readily available to those skilled in the art. See, for example, Molecular Cloning, A Laboratory Manual, 3rd edition, edited by Sambrook, J., MacCallum, P., and Russell, D.W. (2001), Cold Spring Harbor Laboratory Press (ISBN: 0-879-69576-5); Current
20 Protocols In Molecular Biology, edited by Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Struhl, K. (1993), John Wiley and Sons, Inc. (ISBN: 0-471-30661-4); PCR Applications: Protocols for Functional Genomics, edited by Innis, M.A., Gelfand, D.H., Sninsky, J.J. (1999), Cold Spring Harbor Press (ISBN: 0-123-72186-5); and Methods In Yeast Genetics, 2000 Edition: A Cold Spring Habor Laboratory Course Manual, by Burke, D., Dawson, D. and Stearns, T., Cold Spring Harbor Press (ISBN: 0-879-69588-9).

30 In certain embodiments of the aspects of the invention, the invention relates to the biosynthesis and improved production of secondary metabolites. The invention provides variant regulator proteins useful for the production of secondary metabolites, nucleic acid
35 molecules encoding variant regulator proteins, and methods for their production.

In a first aspect, the invention provides a variant regulator protein of secondary metabolite production with increased activity relative to a cognate, wild-type

5 regulator protein. Particularly preferred are variant
regulator proteins of fungal secondary metabolites.

As used herein, the terms "fungal" and "fungus"
refer generally to eukaryotic, heterotrophic organisms
with an absorptive mode of nutrition. Fungi typically
10 contain chitin in their cell walls and exhibit mycelial
or yeast-like growth habits (More Gene Manipulations in
Fungi, edited by J.W. Bennet and L.L. Lasure, Academic
Press Inc. (1991), ISBN 0120886421). More specifically,
the terms refer to secondary metabolite producing
15 organisms including, without limitation, *Aspergillus sp.*,
Penicillium sp., *Acremonium chrysogenum*, *Yarrowia*
lipolytica, *Nodulisporium sp.*, *Fusarium sp.*, *Monascus*
sp., *Claviceps sp.*, *Trichoderma sp.*, *Tolypocladium sp.*,
Tricotheicium sp., *Fusidium sp.*, *Emericellopsis sp.*,
20 *Cephalosporium sp.*, *Cochliobolus sp.*, *Helminthosporium*
sp., *Agaricus brunescens*, *Ustilago maydis*, *Neurospora*
sp., *Pestalotiopsis sp.* and *Phaffia rhodozyma* (See,
Fungal Physiology, Chapter 9 (Secondary(Special)
Metabolism), Griffin, D. H., John Wiley & Sons, Inc.;
25 ISBN: 0471166154).

The term "variant regulator protein" is used herein
to refer to any regulatory protein having at least one
change or difference in the amino acid sequence of the
protein when compared to its cognate, wild-type
30 regulatory protein sequence. The term does not include
naturally occurring allelic variations of the cognate,
wild-type regulatory protein.

The term "regulator protein" is meant to refer to a
protein having a positive or negative function that
35 modifies the production of a secondary metabolite. The
function of the protein may be at the level of
transcription, e.g., repression or activation, protein
synthesis, or transport. The regulator may alter the
level of transcription, RNA stability, translation, post-

5 translational modification, or cellular localization of
proteins involved in secondary metabolite synthesis
and/or transport. The regulator may also have effects on
precursor metabolite pools, flux through specific
pathways and metabolite resistance.

10 By way of non-limiting example, certain embodiments
of the aspects of the invention relate to a regulator
protein that is a protein that contributes and/or
promotes transcription of a gene sequence, i.e., a
transcription-activating protein. "Transcription-
15 activating" is a term used to refer to characteristics of
a protein that promote transcription. As used herein, a
transcription-activating protein would include proteins
that increase accessibility of the DNA to transcription
complexes, for example, by opening or relaxing chromatin
20 structure, proteins that promote the recognition and/or
binding of transcription complexes to a target gene
sequence, and/or proteins that promote transcription
complex movement along the length of the template DNA
sequence.

25 Regulatory proteins of secondary metabolite
production and the nucleic acid sequences encoding these
are known to those skilled in the art. Non-limiting
examples of regulatory proteins of secondary metabolite
synthesis include: regulator proteins of the
30 aflatoxin/sterigmatocystin biosynthetic cluster
(Woloshuk, C.P., et al., *Appl. Environ. Microbiol.*
60:2408-2414 (1994) and Brown, D.W., et al., *Proc Natl*
Acad Sci U S A. *93*:1418-1422 (1996)); regulator proteins
of the paxilline biosynthetic cluster (Young, C., et al.,
35 *Mol. Microbiol.* *39*:754-764 (2001)); regulator proteins of
the cephalosporin and penicillin biosynthetic clusters
(Litzka O., et al., *Antonie Van Leeuwenhoek* *75*:95-105
(1999); Schmitt E.K. and Kuck U., *J. Biol. Chem.*
275:9348-9357 (2000); MacCabe et al. *Mol. Gen. Genet.*

- 5 250:367-374 (1996); Suarez et al. *Mol. Microbiol.*
 20:529-540 (1996); Lambert et al. *Mol. Cell. Biol.*
 17:3966-3976 (1997); Su et al. *Genetics* 133:67-77 (1993);
 regulator proteins of tricothecene synthesis (Trapp S.C.,
 et al., *Mol. Gen. Genet.* 257:421-432 (1998); Brown D.W.,
 10 et al., *Fungal Genet. Biol.* 32:121-133 (2001); and
 Matsumoto G., et al. *Biosci. Biotechnol. Biochem.*
 63:2001-2004 (1999)); and regulator proteins of
 lovastatin synthesis (Kennedy, J., et al., *Science*
 284:1368-1372 (1999); Hendrickson et al., *Chem. Biol.*
 15 6:429-439 (1999) Tag, A. et al., *Mol Microbiol.* 38:658-65
 (2000)).

Certain embodiments of the aspects of the invention
 disclosed herein relate to the lovE regulator protein, a
 protein which plays a key role in the biosynthesis of
 20 lovastatin. More particularly, certain embodiments of
 the aspects of the invention relate to variant proteins
 of the lovE regulator protein and methods of making the
 same. Such proteins are variant with respect to the
 following *A. terreus* wild-type lovE sequences (SEQ ID
 25 NOS:91 and 92).

Table 1: Amino Acid and Nucleic Acid Sequences of Wild-type lovE	
Wild-type lovE Amino Acid Sequence	
maadggiftnsvtlspvegsrtggtlprrafrrrscdrchaqkikctgnkevtgrapcqrc qqaglrvcysercpkrklrqsraadlvsadpdpclhmssppvpvpsqslpldvsseshsnts rqfldppdsydwswtsigtdeaidtdcwglsgcdggfscqleptlpdlpspfestvekap lppvssdiaraasaqrelfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrl ltvlrqqaqadchqgtldeclrtknlfavhcyilnvrltaiselllsqirrtqnshms plegsrqspsrddtssssghssvdtipffsenlpigelfsyvdplthalfsacttlhvg vqllreneitlgvhsaggiaasismsgepgediartgatnsarceeqpttpaarvlfmfl sdegafqeaksagsrgrtiaalrrcyedifslarkhkhgmlrdlnnipp (SEQ ID NO:91)	
Wild-type lovE DNA Sequence	
atggctgcagatcaaggtatatattcacgaactcggtcactctctcgccagtggagggttca cgcaccggtggaacattaccccgccgtgcattccgacgctcttgatcggtgtcatgca caaaagatcaaagtactggaaataaggaggttactggccgtgctccctgtcagcggttgc cagcaggctggacttcgatgcgtctacagtgcgatgccccaaagcgcaagctacgcaa tccagggcagcggatctcgtctctgctgaccagatccctgcttgacatgtcctcgct ccagtgccctcacagagcttgccgctagacgtatccgagtcgcattcctcaaatacctcc cggcaatttcttgatccaccggacagctacgactggctcgtggacctcgattggcactgac	

gaggctattgacactgactgctgggggctgtcccaatgtgatggaggcttcagctgtcag
 ttagagccaacgctgccggatctaccttcgcccttcgagctctacgggtgaaaaagctccg
 ttgccaccggtatcgagcgacattgctcgtgcgggccagtgcgcaacgagagcttttcgat
 gacctgtcggcggtgtcgcaggaactggaagagatccttctggccgtgacggtagaatgg
 ccgaagcaggaaatctggacccatcccatcggaatgtttttcaatgctgcacgacggctt
 cttactgtcctgcgccaacaagcgcaggccgactgccatcaaggcacactagacgaatgt
 ttacggaccaagaacctctttacggcagtagactgttacatattgaatgtgcggattttg
 accgccatatcggagttgctcctgtcgcaaattaggcggacccagaacagccatatgagc
 ccactggaagggagtcgatcccagtcgccgagcagagacgacaccagcagcagcagcggc
 cacagcagtggttgacaccatacccttcttttagcgagaacctccctattggtgagctgttc
 tctatgttgacccccctgacacacgccttattctcggcttgactacgttacatgttggg
 gtacaattgctgcgtgagaatgagattactctgggagtacactccgcccaggggcattgca
 gcttccatcagcatgagcggggaaccaggcgaggatatagccaggacagggggcgaccaat
 tccgcaagatgcgaggagcagccgaccactccagcggctcgggttttgttcatgttcttg
 agtgatgaaggggctttccaggaggcaaagtctgctgggttcccagggtcgaaccatcgca
 gcaactgcgacgatgctatgaggatatcttttccctcgcccgaacacaaaacatggcatg
 ctcagagacctcaacaatatctcctccatga (SEQ ID NO:92)

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As used herein, the term "secondary metabolite" means a compound, derived from primary metabolites, that is produced by an organism, is not a primary metabolite, is not ethanol or a fusel alcohol, and is not required
 10 for growth under standard conditions. Secondary metabolites are derived from intermediates of many pathways of primary metabolism. These pathways include, without limitation, pathways for biosynthesis of amino acids, the shikimic acid pathway for biosynthesis of
 15 aromatic amino acids, the polyketide biosynthetic pathway from acetyl coenzyme A (CoA), the mevalonic acid pathway from acetyl CoA, and pathways for biosynthesis of polysaccharides and peptidopolysaccharides. Collectively, secondary metabolism involves all primary
 20 pathways of carbon metabolism. Particularly preferred in embodiments of the aspects of the invention are fungal secondary metabolites (See, Fungal Physiology, Chapter 9 (Secondary(Special) Metabolism), Griffin, D. H., John Wiley & Sons, Inc.; ISBN: 0471166154).

25 "Secondary metabolite" also includes intermediate compounds in the biosynthetic pathway for a secondary metabolite that are dedicated to the pathway for

5 synthesis of the secondary metabolite. "Dedicated to the
 pathway for synthesis of the secondary metabolite" means
 that once the intermediate is synthesized by the cell,
 the cell will not convert the intermediate to a primary
 metabolite. "Intermediate compounds" also include
 10 secondary metabolite intermediate compounds which can be
 converted to useful compounds by subsequent chemical
 conversion or subsequent biotransformation. As such,
 providing improved availability of such intermediate
 compounds would still lead to improved production of the
 15 ultimate useful compound, which itself may be referred to
 herein as a secondary metabolite. The yeast
Saccharomyces cerevisiae is not known to produce
 secondary metabolites.

The term "primary metabolite" means a natural
 20 product that has an obvious role in the functioning of
 almost all organisms. Primary metabolites include,
 without limitation, compounds involved in the
 biosynthesis of lipids, carbohydrates, proteins, and
 nucleic acids. The term "increasing the yield of the
 25 secondary metabolite" means increasing the quantity of
 the secondary metabolite present in the total
 fermentation broth per unit volume of fermentation broth
 or culture.

As used herein, the phrase "modulate production of a
 30 secondary metabolite" refers to a positive or negative or
 desirable change in one or more of the variables or
 values that affect the process or results of production
 of the primary or secondary metabolites in a liquid or
 solid state fungal fermentation. These positive or
 35 negative or desirable changes include, without
 limitation, an increase or decrease in the amount of a
 primary or secondary metabolite being produced (in
 absolute terms or in quantity per unit volume of
 fermentation broth or per unit mass of solid substrate);

5 a decrease in the volume of the broth or the
 mass/quantity of substrate required for the production of
 sufficient quantities; a decrease in the cost of raw
 materials and energy, the time of fermentor or culture
 run, or the amount of waste that must be processed after
 10 a fermentor run; an increase or decrease in the specific
 production of the desired metabolite (both in total
 amounts and as a fraction of all metabolites and side
 products made by the fungus); an increase or decrease in
 the percent of the produced secondary metabolite that can
 15 be recovered from the fermentation broth or culture; and
 an increase in the resistance of an organism producing a
 primary or secondary metabolite to possible deleterious
 effects of contact with the secondary metabolite.

In certain embodiments of aspects of the invention,
 20 a secondary metabolite is an anti-bacterial. An "anti-
 bacterial" is a molecule that has cytotoxic or cytostatic
 activity against some or all bacteria. Preferred anti-
 bacterials include, without limitation, β -lactams.
 Preferred β -lactams include, without limitation,
 25 penicillins and cephalosporins and biosynthetic
 intermediates thereof. Preferred penicillins and
 biosynthetic intermediates include, without limitation,
 isopenicillin N, 6-aminopenicillanic acid (6-APA),
 penicillin G, penicillin N, and penicillin V. Preferred
 30 cephalosporins and biosynthetic intermediates include,
 without limitation, deacetoxycephalosporin V (DAOC V),
 deacetoxycephalosporin C (DAOC), deacetylcephalosporin C
 (DAC), 7-aminodeacetoxycephalosporanic acid (7-ADCA),
 cephalosporin C, 7-B-(5-carboxy-5-oxopentanamido)-
 35 cephalosporanic acid (keto-AD-7ACA), 7-B-(4-
 carboxybutanamido)-cephalosporanic acid (GL-7ACA), and 7-
 aminocephalosporanic acid (7ACA).

5 In certain embodiments of aspects of the invention,
the secondary metabolite is an anti-hypercholesterolemic
or a biosynthetic intermediate thereof. An "anti-
hypercholesterolemic" is a drug administered to a patient
diagnosed with elevated cholesterol levels for the
10 purpose of lowering the cholesterol levels. Preferred
anti-hypercholesterolemic include, without limitation,
lovastatin, mevastatin, simvastatin, and pravastatin.

According to other embodiments of the invention, a
secondary metabolite is an immunosuppressant or a
15 biosynthetic intermediate thereof. An
"immunosuppressant" is a molecule that reduces or
eliminates an immune response in a host when the host is
challenged with an immunogenic molecule, including
immunogenic molecules present on transplanted organs,
20 tissues or cells. Preferred immunosuppressants include,
without limitation, members of the cyclosporin family and
beauverolide L. Preferred cyclosporins include, without
limitation, cyclosporin A and cyclosporin C.

In certain embodiments of aspects of the invention,
25 the secondary metabolite is an ergot alkaloid or a
biosynthetic intermediate thereof. An "ergot alkaloid"
is a member of a large family of alkaloid compounds that
are most often produced in the sclerotia of fungi of the
genus Claviceps. An "alkaloid" is a small molecule that
30 contains nitrogen and has basic pH characteristics. The
classes of ergot alkaloids include clavine alkaloids,
lysergic acids, lysergic acid amides, and ergot peptide
alkaloids. Preferred ergot alkaloids include, without
limitation, ergotamine, ergosine, ergocristine,
35 ergocryptine, ergocornine, ergotaminine, ergosinine,
ergocristinine, ergocryptinine, ergocorninine,
ergonovine, ergometrinine, and ergoclavine.

In certain embodiments of aspects of the invention,
the secondary metabolite is an inhibitor of angiogenesis

5 or a biosynthetic intermediate thereof. An "angiogenesis inhibitor" is a molecule that decreases or prevents the formation of new blood vessels. Angiogenesis inhibitors have proven effective in the treatment of several human diseases including, without limitation, cancer,
10 rheumatoid arthritis, and diabetic retinopathy. Preferred inhibitors of angiogenesis include, without limitation, fumagillin and ovalicin.

In certain embodiments of aspects of the invention, the secondary metabolite is a glucan synthase inhibitor
15 or a biosynthetic intermediate thereof. A "glucan synthase inhibitor" is a molecule that decreases or inhibits the production of 1,3- β -D-glucan, a structural polymer of fungal cell walls. Glucan synthase inhibitors are a class of antifungal agents. Preferred glucan
20 synthase inhibitors include, without limitation, echinocandin B, pneumocandin B, aculeacin A, and papulacandin.

In certain embodiments of aspects of the invention, the secondary metabolite is a member of the gliotoxin
25 family of compounds or a biosynthetic intermediate thereof. The "gliotoxin family of compounds" are related molecules of the epipolythiodioxopiperazine class. Gliotoxins display diverse biological activities, including, without limitation, antimicrobial, antifungal,
30 antiviral, and immunomodulating activities. Preferred members of the "gliotoxin family of compounds" include, without limitation, gliotoxin and aspirochlorine.

In certain embodiments of aspects of the invention, the secondary metabolite is a fungal toxin or a
35 biosynthetic intermediate thereof. A "fungal toxin" is a compound that causes a pathological condition in a host, either plant or animal. Fungal toxins could be mycotoxins present in food products, toxins produced by

5 phytopathogens, toxins from poisonous mushrooms, or
toxins produced by zoopathogens. Preferred fungal toxins
include, without limitation, aflatoxins, patulin,
zearalenone, cytochalasin, griseofulvin, ergochrome,
cercosporin, marticin, xanthocillin, coumarins,
10 tricothecenes, fusidanes, sesterpenes, amatoxins,
malformin A, phallotoxins, pentoxin, HC toxin,
psilocybin, bufotenine, lysergic acid, sporodesmin,
pulcheriminic acid, sordarins, fumonisins, ochratoxin A,
and fusaric acid.

15 With some certain embodiments of aspects of the invention, the secondary metabolite is a modulator of cell surface receptor signaling or a biosynthetic intermediate thereof. The term "cell surface receptor" is as used before. Modulators of cell surface receptor
20 signaling might function by one of several mechanisms including, without limitation, acting as agonists or antagonists, sequestering a molecule that interacts with a receptor such as a ligand, or stabilizing the interaction of a receptor and molecule with which it
25 interacts. Preferred modulators of cell surface signaling include, without limitation, the insulin receptor agonist L-783,281 and the cholecystokinin receptor antagonist asperlicin.

In certain embodiments of aspects of the invention, the secondary metabolite is a plant growth regulator or a biosynthetic intermediate thereof. A "plant growth regulator" is a molecule that controls growth and development of a plant by affecting processes that include, without limitation, division, elongation, and differentiation of cells. Preferred plant growth regulators include, without limitation, cytokinin, auxin, gibberellin, abscisic acid, and ethylene.

In certain embodiments of aspects of the invention, the secondary metabolite is a pigment or a biosynthetic

5 intermediate thereof. A "pigment" is a substance that imparts a characteristic color. Preferred pigments include, without limitation, melanins and carotenoids.

In certain embodiments of aspects of the invention, the secondary metabolite is an insecticide or a
10 biosynthetic intermediate thereof. An "insecticide" is a molecule that is toxic to insects. Preferred insecticides include, without limitation, nodulisporic acid.

In certain embodiments of aspects of the invention, the secondary metabolite is an anti-neoplastic compound
15 or a biosynthetic intermediate thereof. An "anti-neoplastic" compound is a molecule that prevents or reduces tumor formation. Preferred anti-neoplastic compounds include, without limitation, taxol (paclitaxel)
20 and related taxoids.

The phrase "increased activity" is used herein to refer to a characteristic that results in an augmentation of the inherent negative or positive function of the regulatory protein.

25 The invention provides variant regulator proteins of secondary metabolite production with increased activity and methods of producing the same. The invention further provides for the identification of specific amino acid residues that are important to the functioning of
30 secondary metabolite regulator proteins. By way of non-limiting example, variant regulator proteins of the secondary metabolite regulator lovE are presented herein.

As known to those skilled in the art, certain substitutions of one amino acid for another may be
35 tolerated at one or more amino acid residues of a wild-type regulator protein absent a change in the structure, activity and/or function of the wild-type protein. Such substitutions are referred to in the art as "conservative" substitutions, and amino acids may be

5 categorized into groups that identify which amino acids
may be substituted for another without altering the
structure and/or function of the protein.

As used herein, the term "conservative substitution"
refers to the exchange of one amino acid for another in
10 the same conservative substitution grouping in a protein
sequence. Conservative amino acid substitutions are
known in the art and are generally based on the relative
similarity of the amino acid side-chain substituents, for
example, their hydrophobicity, hydrophilicity, charge,
15 size, and the like. In a preferred embodiment,
conservative substitutions typically include
substitutions within the following groups: Group 1:
glycine, alanine, and proline; Group 2: valine,
isoleucine, leucine, and methionine; Group 3: aspartic
20 acid, glutamic acid, asparagine, glutamine; Group 4:
serine, threonine, and cysteine; Group 5: lysine,
arginine, and histidine; Group 6: phenylalanine,
tyrosine, and tryptophan. Each group provides a listing
of amino acids that may be substituted in a protein
25 sequence for any one of the other amino acids in that
particular group.

As stated *supra*, there are several criteria used to
establish groupings of amino acids for conservative
substitution. For example, the importance of the
30 hydropathic amino acid index in conferring interactive
biological function on a protein is generally understood
in the art (Kyte and Doolittle, *Mol. Biol.* 157:105-132
(1982)). It is known that certain amino acids may be
substituted for other amino acids having a similar
35 hydropathic index or score and still retain a similar
biological activity. Amino acid hydrophilicity is also
used as a criteria for the establishment of conservative
amino acid groupings (see, e.g., U.S. Patent No.
4,554,101).

5 Information relating to the substitution of one
 amino acid for another is generally known in the art
 (see, e.g., Introduction to Protein Architecture : The
Structural Biology of Proteins, Lesk, A.M., Oxford
 University Press; ISBN: 0198504748; Introduction to
 10 Protein Structure, Branden, C.-I., Tooze, J., Karolinska
 Institute, Stockholm, Sweden (January 15, 1999); and
Protein Structure Prediction: Methods and Protocols
(Methods in Molecular Biology), Webster, D.M. (Editor),
 August 2000, Humana Press, ISBN: 0896036375).

15 In one embodiment of the first aspect, the invention
 provides an improved regulator protein comprising an
 amino acid sequence coding for a variant of the lovE
 protein having at least one specific mutation that gives
 rise to greater transcription-activating properties of
 20 the regulator protein and/or increased lovastatin
 synthesis.

By way of non-limiting example, certain amino acid
 residues and mutations thereof in the lovE regulatory
 protein of *A. terreus* (SEQ ID NO:91) are identified by
 25 the invention described herein. Mutations at residues
 31, 41, 52, 73, 101, 111, 133, 141, 153, 281, 367, and
 389 of the wild-type lovE protein of *A. terreus* have been
 identified as being critical for the improvement of lovE
 regulator protein function. Those mutations include:
 30 F31L, Q41K, Q41R, T52I, T52N, C73R, P101S, P101Q, V111I,
 S133L, E141V, E141K, C153Y, C153R, T281A, N367I, N367Y,
 P389S and P389L. Each mutation, therefore, represents a
 change of one conservative class of amino acids for
 another. For example, the mutation F31L represents a
 35 change from a Group 6 amino acid residue to a Group 2
 amino acid residue at position 31 of the wild-type, lovE
 regulator protein.

Thus, by way of non-limiting example, regulator
 proteins of this aspect of the invention include at least

5 one of the following mutations: (1) a Group 6 amino acid
residue mutated to a Group 2 amino acid residue at
position 31, for example, the mutation represented by
F31L; (2) a Group 3 amino acid residue mutated to a Group
5 amino acid residue at position 41, for example, the
10 mutation represented by Q41K or Q41R; (3) a Group 4 amino
acid residue mutated to a Group 2 amino acid residue at
position 52, for example, the mutation represented by
T52I; (4) a Group 4 amino acid residue mutated to a Group
3 amino acid residue at position 52, for example, the
15 mutation represented by T52N; (5) a Group 4 amino acid
residue mutated to a Group 5 amino acid residue at
position 73, for example, the mutation represented by
C73R; (6) a Group 1 amino acid residue mutated to a Group
4 amino acid residue at position 101, for example, the
20 mutation represented by P101S; (7) a Group 1 amino acid
residue mutated to a Group 3 amino acid residue at
position 101, for example, the mutation represented by
P101Q; (8) a valine amino acid residue mutated to another
Group 2 amino acid residue at position 111, for example,
25 the mutation represented by V111I; (9) a Group 4 amino
acid residue mutated to a Group 2 amino acid residue at
position 133, for example, the mutation represented by
S133L; (10) a Group 3 amino acid residue mutated to a
Group 2 amino acid residue at position 141, for example,
30 the mutation represented by E141V; (11) a Group 3 amino
acid residue mutated to a Group 5 amino acid residue at
position 141, for example, the mutation represented by
E141K; (12) a Group 4 amino acid residue mutated to Group
6 amino acid residue at position 153, for example, the
35 mutation represented by C153Y; (13) a Group 4 amino acid
residue mutated to a Group 5 amino acid residue at
position 153, for example, the mutation represented by
C153R; (14) a Group 4 amino acid residue mutated to a
Group 1 amino acid residue at position 281, for example,

5 the mutation represented by T281A; (15) a Group 3 amino acid residue mutated to a Group 2 amino acid residue at position 367, for example, the mutation represented by N367I; (16) a Group 3 amino acid residue mutated to a Group 6 amino acid residue at position 367, for example,
 10 the mutation represented by N367Y; (17) a Group 1 amino acid residue mutated to Group 4 amino acid residue at position 389, for example, the mutation represented by P389S; and/or (18) a Group 1 amino acid residue mutated to a Group 2 amino acid residue at position 389, for
 15 example, the mutation represented by P389L.

In other embodiments of the first aspect, the invention provides a variant of the lovE regulator protein with at least two, or at least three, or at least four, or at least five, or at least six, or at least
 20 seven, or at least eight, or at least nine, or at least ten, or at least eleven, or at least twelve, or at least thirteen, or at least fourteen, or at least fifteen, or at least sixteen, or at least seventeen, or at least eighteen of the above described specific mutations.

25 In other embodiments of the first aspect, the invention provides an isolated lovE variant regulator protein having the sequence of SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID
 30 NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, or SEQ ID NO:65.

In a second aspect, the invention provides a nucleic
 35 acid molecule encoding a variant regulator protein of secondary metabolite production of the first aspect of the invention. As used herein, the terms "nucleic acid" or "nucleic acid molecule" refer to a deoxyribonucleotide or ribonucleotide polymer in either single-or double-

5 Other genetically tractable organisms could also be used for this purpose.

In a third aspect, the invention provides a method of increasing the activity of a protein that regulates secondary metabolite production comprising: (a) selecting
10 a nucleic acid comprising a polynucleotide encoding a protein regulator of secondary metabolite production; (b) mutating the nucleic acid to create a plurality of nucleic acid molecules encoding variant regulator proteins of secondary metabolite production; and (c)
15 selecting a variant regulator protein with more activity than the cognate, wild-type protein.

As used herein, "mutating" is used to refer to the deliberate alteration of at least one nucleotide residue of a wild-type, cognate nucleic acid sequence encoding a regulator protein of secondary metabolite production. A
20 deliberate alteration or change in at least one nucleotide residue of a polynucleotide may be accomplished by any method known in the art. The mutation(s) can be made *in vivo* or *in vitro* and can
25 include random, partially random or not random, *i.e.*, directed, mutagenesis techniques.

By way of non-limiting example, *in vivo* mutagenesis can be done by placing this nucleic acid molecule in a cell with a high mutation frequency, *i.e.* a mutagenic
30 strain. By way of non-limiting example, Muhlrad et al. (Yeast 8:79-82 (1992)) have developed a rapid method for localized mutagenesis of yeast genes. As a first step, the region of interest of a gene sequence is first amplified *in vitro* under error-prone polymerase chain
35 reaction (PCR) conditions. Error-prone polymerase chain reaction (PCR) is a method of introducing amino acid changes into proteins. With this technique, mutations are deliberately introduced during the PCR reaction through the use of error-prone DNA polymerases under

5 specific reaction conditions. With the Muhlrad et al. procedure, the PCR product is then co-transformed with a gapped plasmid containing homology to both ends of the PCR product, resulting in *in vivo* recombination to repair the gap with the mutagenized DNA.

10 There are a variety of commercially available kits that may be used to produce mutant nucleic acid molecules by error-prone PCR (see, e.g., GeneMorph™ PCR Mutagenesis Kit (Stratagene, La Jolla, California); and Diversify™ PCR Random Mutagenesis Kit (BD Biosciences Clontech, Palo Alto, CA). Thus, a plurality of variant, *i.e.*, mutated, regulator proteins of secondary metabolite production may be produced using established mutagenesis techniques.

As used herein, the term "activity" refers to a characteristic of the regulator protein that negatively or positively affects the biological system to bring about a modulation in secondary metabolite production. By way of non-limiting example, the activity is the transcription of downstream genes involved in the biosynthetic pathway of the secondary metabolite of choice. Thus, in the present example, the phrase "more activity" refers to the property of a variant regulator protein to bring about more transcription than that effected by the cognate, wild-type regulator protein.

In certain embodiments of the third aspect, the selected variant regulator protein has more activity in a fungal cell than the cognate, wild-type protein. In certain embodiments of the third aspect, the protein regulator of secondary metabolite production is a transcription factor. In certain embodiments of the fourth aspect, the protein regulator of secondary metabolite production is a transmembrane transporter, a protein that mediates secretion, a kinase, a G-protein, a cell surface receptor, a GTPase activating protein, a

5 guanine nucleotide exchange factor, a phosphatase, a
 protease, a phosphodiesterase, a bacterial protein toxin,
 an importin, an RNA-binding protein, an SCF complex
 component, an adherin, or a protein encoded within a
 biosynthetic cluster. . In certain other embodiments of
 10 the third aspect, the selected variant regulator protein
 has more activity in a heterologous cell than the
 cognate, wild-type protein. In certain embodiments
 thereof, the heterologous cell is an organism selected
 from the group consisting of *S. cerevisiae*, *E. coli*, *A.*
 15 *nidulans*, *Candida sp.*, and *N. crassa*. In yet certain
 other embodiments of the third aspect, the selected
 variant regulator protein has more activity in a
 homologous cell than the cognate, wild-type protein. In
 certain embodiments thereof, the homologous cell is an
 20 organism selected from the group consisting of
Aspergillus sp., *Penicillium sp.*, *Acremonium chrysogenum*,
Yarrowia lipolytica, *Nodulisporium sp.*, *Fusarium sp.*,
Monascus sp., *Claviceps sp.*, *Trichoderma sp.*,
Tolypocladium sp., *Tricotheicium sp.*, *Fusidium sp.*,
 25 *Emericellopsis sp.*, *Cephalosporium sp.*, *Cochliobolus sp.*,
Helminthosporium sp., *Agaricus brunescens*, *Ustilago*
maydis, *Neurospora sp.*, *Pestalotiopsis sp.*, and *Phaffia*
rhodozyma.

In certain embodiments of the third aspect, the
 30 selected variant regulator protein has more activity in a
 heterologous cell and a homologous cell than the cognate,
 wild-type protein. In certain embodiments thereof, the
 heterologous cell is an organism selected from the group
 consisting of *S. cerevisiae*, *E. coli*, *A. nidulans*,
 35 *Candida sp.*, and *N. crassa* and the homologous cell is an
 organism selected from the group consisting of
Aspergillus sp., *Penicillium sp.*, *Acremonium chrysogenum*,

5 *Yarrowia lipolytica*, *Nodulisporium* sp., *Fusarium* sp.,
Monascus sp., *Claviceps* sp., *Trichoderma* sp.,
Tolypocladium sp., *Tricotheicium* sp., *Fusidium* sp.,
Emericellopsis sp., *Cephalosporium* sp., *Cochliobolus* sp.,
Helminthosporium sp., *Agaricus brunescens*, *Ustilago*
10 *maydis*, *Neurospora* sp., *Pestalotiopsis* sp. and *Phaffia*
rhodozyma.

As used herein, the phrase "heterologous cell"
refers to a system for gene expression, i.e., an organism
for gene expression, that is one other than the organism
15 from which the selected regulator protein of secondary
metabolite production has been isolated. Preferred
heterologous cells include, but are not limited to, *S.*
cerevisiae, *E. coli*, *A. nidulans*, and *Candida* sp., and
N. crassa. Particularly preferred are fungal
20 heterologous cells. In an embodiment of the third
aspect, the method comprises: (a) selecting a nucleic
acid comprising a polynucleotide encoding a protein
regulator of secondary metabolite production; (b)
mutating the nucleic acid to create a plurality of
25 nucleic acid molecules encoding variant regulator
proteins of secondary metabolite production; and (c)
selecting a mutagenized nucleic acid encoding a variant
regulator protein with increased activity in a homologous
cell than the cognate, wild-type protein.

30 As used herein, the phrase "homologous cell" refers
to a system for gene expression, i.e., an organism for
gene expression, that is the organism from which the
regulator protein of secondary metabolite production has
been isolated. Preferred homologous cells are fungal
35 homologous cells, including, but not limited to,
Aspergillus sp., *Penicillium* sp., *Acremonium chrysogenum*,
Yarrowia lipolytica, *Nodulisporium* sp., *Fusarium* sp.,
Monascus sp., *Claviceps* sp., *Trichoderma* sp.,

- 5 *Tolypocladium sp.*, *Tricotheicum sp.*, *Fusidium sp.*,
Emericellopsis sp., *Cephalosporium sp.*, *Cochliobolus sp.*,
Helminthosporium sp., *Agaricus brunescens*, *Ustilago*
maydis, *Neurospora sp.*, *Pestalotiopsis sp* and *Phaffia*
rhodozyma. (See, Fungal Physiology, Chapter 9
10 (Secondary(Special) Metabolism), Griffin, D. H., John
Wiley & Sons, Inc.; ISBN: 0471166154).

In certain embodiments of the third aspect, the
method further comprises selecting a variant regulator
protein that also increases production of a secondary
15 metabolite in a cell when compared to the cognate, wild-
type protein. In certain embodiments thereof, the cell
is a fungal cell. In certain embodiments thereof, the
cell is a heterologous cell, preferably selected from the
group consisting of *S. cerevisiae*, *E. coli*, *A. nidulans*,
20 *Candida sp.*, and *N. crassa*.

In certain embodiments thereof, the cell is a
homologous cell, preferably selected from the group
consisting of *Aspergillus sp.*, *Penicillium sp.*,
Acremonium chrysogenum, *Yarrowia lipolytica*,
25 *Nodulisporium sp.*, *Fusarium sp.*, *Monascus sp.*, *Claviceps*
sp., *Trichoderma sp.*, *Tolypocladium sp.*, *Tricotheicum*
sp., *Fusidium sp.*, *Emericellopsis sp.*, *Cephalosporium*
sp., *Cochliobolus sp.*, *Helminthosporium sp.*, *Agaricus*
brunescens, *Ustilago maydis*, *Neurospora sp.*,
30 *Pestalotiopsis sp.*, and *Phaffia rhodozyma*.

Certain embodiments of the aspects of the invention
relate to regulator proteins that promote secondary
metabolite production by increasing transcription of one
or more genes involved with secondary metabolite
35 production. These wild-type sequences may be selected
for mutagenesis to create a plurality of variant
regulator proteins. The activity of these transcription-

5 activating variant regulator proteins may be determined
by measuring the activity of a reporter gene having the
appropriate promoter sequences. These tests are done in
a homologous and/or a heterologous cell. Certain
embodiments of aspects of the invention are directed to
10 fungal regulator proteins with transcription-activating
activity that is tested in fungal heterologous and
homologous cells.

Reporter genes are useful for isolating
transformants expressing improved variant regulator
15 proteins. The reporter genes may be operably linked to a
promoter sequence that is normally regulated by the wild-
type regulator protein. Reporter genes include, but are
not limited to, genes encoding β -galactosidase (*lacZ*), β -
glucoronidase (*GUS*), β -glucosidase, amylase and
20 invertase, amino acid biosynthetic genes, e.g., the yeast
LEU2, *HIS3*, *LYS2*, *TRP1* genes (or homologous genes from
other fungi, such as filamentous fungi, that encode
proteins with the similar functional activities), nucleic
acid biosynthetic genes, e.g., the yeast *URA3* and *ADE2*
25 genes (or homologous genes from other fungi, such as
filamentous fungi, that encode proteins with the similar
functional activities), the mammalian chloramphenicol
transacetylase (CAT) gene, or any surface antigen gene
for which specific antibodies are available. A reporter
30 gene can also be a neomycin phosphotransferase(neo) gene,
which encodes neomycin, kanamycin resistance gene and
G418 (geneticin) resistance gene. A reporter gene may
encode a protein detectable by luminescence or
fluorescence, such as green fluorescent protein (GFP).
35 Reporter genes may additionally or alternatively encode
any protein that provides a phenotypic marker, for
example, a protein that is necessary for cell growth or
viability, or a toxic protein that causes cell death.

5 Alternatively, the reporter gene may encode a protein detectable by a color assay leading to the presence or absence of color.

The choice of reporter gene will depend on the type of cell to be transformed. Preferred reporter genes are those that are operable in fungal cells. It is preferable to have two reporter genes within the cell. One reporter gene, when expressed, provides a growth advantage to transformed cells that are expressing the variant regulator protein. This allows for the isolation of such transformants through selective pressures. The other reporter gene provides a colorimetric marker, such as the *lacZ* gene and its encoded protein, β -galactosidase. Alternatively, the second reporter provides a fluorescent or luminescent marker, such as green fluorescent protein (GFP).

In a fourth aspect, the invention provides a method of increasing production of a secondary metabolite comprising: (a) selecting a nucleic acid comprising a polynucleotide encoding a protein regulator of secondary metabolite production; (b) mutating the nucleic acid to create a plurality of nucleic acid molecules encoding variant regulator proteins of secondary metabolite production; (c) selecting a variant regulator protein with more activity than the cognate, wild-type protein; and (d) expressing the selected variant regulator protein in a cell, thereby increasing production of the secondary metabolite in the cell.

In certain embodiments of the fourth aspect, the cell is a fungal cell. In certain embodiments of the fourth aspect, the protein regulator of secondary metabolite production is a transcription factor. In certain embodiments of the fourth aspect, the protein regulator of secondary metabolite production is a

5 transmembrane transporter, a protein that mediates
secretion, a kinase, a G-protein, a cell surface
receptor, a GTPase activating protein, a guanine
nucleotide exchange factor, a phosphatase, a protease, a
phosphodiesterase, a bacterial protein toxin, an
10 importin, an RNA-binding protein, an SCF complex
component, an adherin, or a protein encoded within a
biosynthetic cluster. In certain embodiments of the
fourth aspect, the cell is a heterologous cell,
preferably selected from the group consisting of *S.*
15 *cerevisiae*, *E. coli*, *A. nidulans*, *Candida sp.*, and *N.*
crassa. In certain other embodiments of the fourth
aspect, the cell is a homologous cell, preferably
selected from the group consisting of *Aspergillus sp.*,
Penicillium sp., *Acremonium chrysogenum*, *Yarrowia*
20 *lipolytica*, *Nodulisporium sp.*, *Fusarium sp.*, *Monascus*
sp., *Claviceps sp.*, *Trichoderma sp.*, *Tolypocladium sp.*,
Tricotheicium sp., *Fusidium sp.*, *Emericellopsis sp.*,
Cephalosporium sp., *Cochliobolus sp.*, *Helminthosporium*
sp., *Agaricus brunescens*, *Ustilago maydis*, *Neurospora*
25 *sp.*, *Pestalotiopsis sp.*, and *Phaffia rhodozyma*.

In certain other embodiments of the fourth aspect,
the cell is a heterologous cell and the method further
comprises expressing the variant regulator protein in a
homologous cell, thereby increasing secondary metabolite
30 production in the homologous cell. In certain
embodiments thereof, the heterologous cell is an organism
selected from the group consisting of *S. cerevisiae*, *E.*
coli, *A. nidulans*, *Candida sp.*, , and *N. crassa* and the
homologous cell is an organism selected from the group
35 consisting of *Aspergillus sp.*, *Penicillium sp.*,
Acremonium chrysogenum, *Yarrowia lipolytica*,
Nodulisporium sp., *Fusarium sp.*, *Monascus sp.*, *Claviceps*

5 *sp.*, *Trichoderma sp.*, *Tolypocladium sp.*, *Tricotheicium*
sp., *Fusidium sp.*, *Emericellopsis sp.*, *Cephalosporium*
sp., *Cochliobolus sp.*, *Helminthosporium sp.*, *Agaricus*
brunescens, *Ustilago maydis*, *Neurospora sp.*,
Pestalotiopsis sp. and *Phaffia rhodozyma*.

10 In a fifth aspect, the invention provides an
isolated variant regulator protein of secondary
metabolite production having increased activity compared
to a cognate, wild-type protein, made by the process
comprising: (a) selecting a nucleic acid comprising a
15 polynucleotide encoding a protein regulator of secondary
metabolite production; (b) mutating the nucleic acid to
create a plurality of nucleic acid molecules encoding
variant regulator proteins of secondary metabolite
production; (c) selecting a variant regulator protein
20 with more activity than the cognate, wild-type protein;
and (d) recovering the selected variant regulator
protein.

In certain embodiments of the fifth aspect, the
variant regulator protein selected has more activity in a
25 fungal cell. In certain embodiments of the fifth aspect,
the protein regulator of secondary metabolite production
is a transcription factor. In certain embodiments of the
fifth aspect, the protein regulator of secondary
metabolite production is a transmembrane transporter, a
30 protein that mediates secretion, a kinase, a G-protein, a
cell surface receptor, a GTPase activating protein, a
guanine nucleotide exchange factor, a phosphatase, a
protease, a phosphodiesterase, a bacterial protein toxin,
an importin, an RNA-binding protein, an SCF complex
35 component, an adherin, or a protein encoded within a
biosynthetic cluster. In certain embodiments of the
fifth aspect, the variant regulator protein selected has

5 more activity in a heterologous cell, preferably selected
 from the group consisting of *S. cerevisiae*, *E. coli*, *A.*
nidulans, *Candida* sp., *Neurospora* sp., *Pestalotiopsis*
 sp., and *N. crassa*. In certain embodiments of the fifth
 aspect, the variant regulator protein selected has more
 10 activity in a homologous cell, preferably selected from
 the group consisting of *Aspergillus* sp., *Penicillium* sp.,
Acremonium chrysogenum, *Yarrowia lipolytica*,
Nodulisporium sp., *Fusarium* sp., *Monascus* sp., *Claviceps*
 sp., *Trichoderma* sp., *Tolypocladium* sp., *Tricotheicum*
 15 sp., *Fusidium* sp., *Emericellopsis* sp., *Cephalosporium*
 sp., *Cochliobolus* sp., *Helminthosporium* sp., *Agaricus*
brunescens, *Ustilago maydis*, *Neurospora* sp.,
Pestalotiopsis sp., and *Phaffia rhodozyma*.

In certain embodiments of the fifth aspect, the
 20 variant regulator protein selected has more activity in a
 homologous cell and a heterologous cell. In embodiments
 thereof, the heterologous cell is an organism selected
 from the group consisting of *S. cerevisiae*, *E. coli*, *A.*
nidulans, *Candida* sp., *Neurospora* sp., *Pestalotiopsis*
 25 sp., and *N. crassa* and the homologous cell is an organism
 selected from the group consisting of *Aspergillus* sp.,
Penicillium sp., *Acremonium chrysogenum*, *Yarrowia*
lipolytica, *Nodulisporium* sp., *Fusarium* sp., *Monascus*
 sp., *Claviceps* sp., *Trichoderma* sp., *Tolypocladium* sp.,
 30 *Tricotheicum* sp., *Fusidium* sp., *Emericellopsis* sp.,
Cephalosporium sp., *Cochliobolus* sp., *Helminthosporium*
 sp., *Agaricus brunescens*, *Ustilago maydis*, *Neurospora*
 sp., *Pestalotiopsis* sp., and *Phaffia rhodozyma*.

In yet another embodiment of the fifth aspect, the
 35 variant regulator protein is a variant protein of the
 lovE protein having at least one of the following
 mutations: (1) a Group 6 amino acid residue mutated to a

5 Group 2 amino acid residue at position 31, for example,
the mutation represented by F31L; (2) a Group 3 amino acid
residue mutated to a Group 5 amino acid residue at
position 41, for example, the mutation represented by
Q41K or Q41R; (3) a Group 4 amino acid residue mutated to
10 a Group 2 amino acid residue at position 52, for example,
the mutation represented by T52I; (4) a Group 4 amino
acid residue mutated to a Group 3 amino acid residue at
position 52, for example, the mutation represented by
T52N; (5) a Group 4 amino acid residue mutated to a Group
15 5 amino acid residue at position 73, for example, the
mutation represented by C73R; (6) a Group 1 amino acid
residue mutated to a Group 4 amino acid residue at
position 101, for example, the mutation represented by
P101S; (7) a Group 1 amino acid residue mutated to a
20 Group 3 amino acid residue at position 101, for example,
the mutation represented by P101Q; (8) a valine amino
acid residue mutated to another Group 2 amino acid
residue at position 111, for example, the mutation
represented by V111I; (9) a Group 4 amino acid residue
25 mutated to a Group 2 amino acid residue at position 133,
for example, the mutation represented by S133L; (10) a
Group 3 amino acid residue mutated to a Group 2 amino
acid residue at position 141, for example, the mutation
represented by E141V; (11) a Group 3 amino acid residue
30 mutated to a Group 5 amino acid residue at position 141,
for example, the mutation represented by E141K; (12) a
Group 4 amino acid residue mutated to Group 6 amino acid
residue at position 153, for example, the mutation
represented by C153Y; (13) a Group 4 amino acid residue
35 mutated to a Group 5 amino acid residue at position 153,
for example, the mutation represented by C153R; (14) a
Group 4 amino acid residue mutated to a Group 1 amino
acid residue at position 281, for example, the mutation
represented by T281A; (15) a Group 3 amino acid residue

5 mutated to a Group 2 amino acid residue at position 367,
for example, the mutation represented by N367I; (16) a
Group 3 amino acid residue mutated to a Group 6 amino
acid residue at position 367, for example, the mutation
represented by N367Y; (17) a Group 1 amino acid residue
10 mutated to Group 4 amino acid residue at position 389,
for example, the mutation represented by P389S; and/or
(18) a Group 1 amino acid residue mutated to a Group 2
amino acid residue at position 389, for example, the
mutation represented by P389L.

15 In certain embodiments of this aspect of the
invention, the variant protein of the lovE protein
sequence has an amino acid sequence of SEQ ID NO:41, SEQ
ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ
ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ
20 ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ
ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ
ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ
ID NO:62, SEQ ID NO:63, SEQ ID NO:64, or SEQ ID NO:65.

In another embodiment thereof, the variant protein
25 of the lovE protein is encoded by a nucleic acid molecule
having a polynucleotide sequence of SEQ ID NO:66, SEQ ID
NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID
NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID
NO:75, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:79, SEQ ID
30 NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID
NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, or SEQ
ID NO:90.

In a sixth aspect, the invention provides a fungus
having improved lovastatin production made by the process
35 of transforming a fungal cell with a nucleic acid
molecule encoding a variant of the lovE protein of the
first aspect of the invention. In an embodiment thereof,
the nucleic acid molecule is selected from a nucleic acid
molecule of the second aspect of the invention.

5 In a seventh aspect, the invention provides an improved process for making lovastatin comprising transforming a fungal cell with a nucleic acid molecule encoding a variant of the lovE protein of the first aspect of the invention. In an embodiment thereof, the
10 fungal cell is transformed with a nucleic acid molecule of the second aspect of the invention.

 International Patent Application PCT/US99/29583 discloses lovastatin production genes. However, this reference does not provide a mature *lovE* cDNA sequence.
15 The invention herein remedies the shortcoming of this reference by providing a complete cDNA sequence for the *lovE* mRNA.

 In an eighth aspect, the invention provides a nucleic acid molecule encoding a *lovE* protein defined by
20 SEQ ID NO:91. In an embodiment thereof, the invention provides an isolated *lovE* nucleic acid molecule defined by SEQ ID NO:92.

 The following examples illustrate the preferred modes of making and practicing the present invention but
25 are not meant to limit the scope of the invention since alternative methods may be utilized to obtain similar results.

EXAMPLES

30

Example 1: Preparation of Strains and Plasmids

 Strain MY2124 was derived from the Sigma 1278b strain background of *S. cerevisiae* and its complete genotype is as follows: *MAT α /MAT α ::LEU2 ura3 Δ 0 /ura3 Δ 0*
35 *leu2 Δ 0/leu2 Δ 0 trp1 Δ 0::hisG/trp1 Δ 0::hisG*
his3 Δ 0::hisG/his3 Δ 0::hisG ura3 Δ 0::lovF-HIS3p-neo/ura3 Δ 0.
MY2124 can be constructed by mating *S. cerevisiae* strains MY2112 (*MAT α ura3 Δ 0 leu2 Δ 0 trp1 Δ 0::hisG his3 Δ 0::hisG*

5 *ura3Δ0::lovFp-HIS3p-neo* with MY1555 (*matα::LEU2 ura3Δ0 leu2Δ0 trp1Δ0::hisG his3Δ0::hisG*) and isolating zygotes. The *ura3Δ0::lovFp-HIS3p-neo* allele of MY2112 was derived by cotransforming *SfiI*-linearized plasmid MB2254 with pRS424 (Sikorski and Hieter (1989) *Genetics* 122:19-27)

10 into MY1413 (*MATα leu2Δ0 trp1Δ0::hisG his3Δ0::hisG*). Transformants were selected on SC-Trp media and subsequently screened for 5-fluoro-orotic acid resistance to identify those transformants containing the *ura3Δ0::lovFp-HIS3p-neo* allele. Trp⁻ segregants lacking

15 plasmid pRS424 were isolated by growing the strain under non-selective conditions.

The following oligonucleotides were used in the construction of plasmids.

Table 2: Oligonucleotides Utilized For <i>LovE</i> Variant Cloning	
MO664	(5' GGCCATGGAGGCCGCTAGCTCGAGTCGACGGCCTAGGTGGCCAGCT3') (SEQ ID NO:1)
MO665	(5' GGCCACCTAGGCCGCTCGACTCGAGCTAGCGGCCTCCATGGCCGTAC3') (SEQ ID NO:2)
MO666	(5' GGCGGCCGCTCTAGAACTAGTCTCGAGGGTACC3') (SEQ ID NO:3)
MO667	(5' GGTACCCTCGAGACTAGTTCTAGAGCGGCCGCC3') (SEQ ID NO:4)
MO1794	(5' CACAGCGCCGCTCAACCTTCCCATTGGGGC3') (SEQ ID NO:5)
MO1793	(5' CACCACTAGTACGCGGGCTGATTCGAC3') (SEQ ID NO:6)
MO1785	(5' CACCACTAGTTATACATTATATAAAGTAATGTG3') (SEQ ID NO:7)
MO1786	(5' CACAGGATCCGTCATCTTTGCCTTCGTTTATC3') (SEQ ID NO:8)
MO195	(5' CGCGGATCCTATTGAACAAGATGGATTGCAC3') (SEQ ID NO:9)
MO196	(5' CCGGAATTCAGAAGAACTCGTCAAGAAG3') (SEQ ID NO:10)
MO841	(5' ACAAAAAGCAGGCTCCACAATGGCTGCAGATCAAGGTAT3') (SEQ ID NO:11)
MO842	(5' ACAAGAAAGCTGGGTTTCATGGAGGAATATTGTTGA3') (SEQ ID NO:12)
MO2278	(5' GGGGATCCAATCGAGGTCCACGACCAGT3') (SEQ ID NO:13)
MO343	(5' GGGGACAAGTTTGTACAAAAAAGCAGGCT3') (SEQ ID NO:14)
MO2273	(5' GGGGATCCGCCAATGGTCCCGTTCAAAC3') (SEQ ID NO:15)
MO2274	(5' ACAAGAAAGCTGGGTTACAGAATGTTTAGCTCAA3') (SEQ ID NO:16)
MO344	(5' GGGGACCACTTTGTACAAAGAAAGCTGGGT3') (SEQ ID NO:17)
MO2624	(5' GCGATGCCCAAGCGCAAGCTACGCCAATCCAGGG3') (SEQ ID NO:18)
MO2654	(5' CGTCGCGCCATTCGCCATTCAGGCTGCGCAACTGT3') (SEQ ID NO:19)

MO2680	(5'GGACCTTTGCAGCATAAATTACTATACTTCT3')	(SEQ ID NO:20)
MO2686	(5'GGCGCGTCCATTTCGCCATTCAGGCTGCGCAACTGT3')	(SEQ ID NO:21)
MO2681	(5'TAAAACTCTTGTTTTCTTCTTTTCTCTAAAT3')	(SEQ ID NO:22)
MO2700	(5'CAGTGAGCGCGCGTAATACGACTCACTATAGGGCGA3')	(SEQ ID NO:23)
MO2701	(5' ATACTTCTATAGACACACAAACACAAATACACACAC3')	(SEQ ID NO:24)
MO107	(5'CGCGGATCCCGTCGTTTTACAAC3')	(SEQ ID NO:25)
MO197	(5'CCCAAGCTTATTATTTTTGACACCAGACCAA3')	(SEQ ID NO:26)
MO1293	(5'GGAAGATCTAGCATCGTGGCCAATTTCTTCTAGTTT3')	(SEQ ID NO:27)
MO1294	(5'ATAAGAATGCGGCCGCTCAACCTTCCCATTGGGGCGTTTGC3')	(SEQ ID NO:28)
MO1787	(5'CACAGGATCCAGCATTATTAATTTAGTGTGTGTATTT3')	(SEQ ID NO:29)
MO1788	(5'CACCACTAGTCTCGAGCAGATCCGCCAG3')	(SEQ ID NO:30)
MO1793	(5'CACCACTAGTACGCGGGCTGATTTCGAC3')	(SEQ ID NO:31)
MO1794	(5'CACAGCGGCCGCTCAACCTTCCCATTGGGGC3')	(SEQ ID NO:32)
MO511	(5'GGCCATCGATACAAGTTTGTACAAAAAGCTGAAC3')	(SEQ ID NO:33)
MO540	(5'GGCGCCCTATTACACCACTTTGTACAAGAAAGC3')	(SEQ ID NO:34)
MO1985	(5'CACACGTCTCCGGCCTCAACCTTCCCATTGGGGCG3')	(SEQ ID NO:35)
MO1986	(5'CACACAGATCTCGTGGCCAATTTCTTCTAGTTTGA3')	(SEQ ID NO:36)
MO1992	(5'CACACGGATCCACAATGTTACGTCCTGTAGAAACCCC3')	(SEQ ID NO:37)
MO1993	(5'CACAGCGGCCGCTTCATTGTTTGCCTCCCTGCTG3')	(SEQ ID NO:38)
MO316	(5'GCGGCCGCGGCGCCCGGCCCATGTCAACAAGAAT3')	(SEQ ID NO:39)
MO318	(5'CCGCGGCCGAGTGGAGATGTGGAGT3')	(SEQ ID NO:40)

5

Plasmid MB2254 contains the *lovFp-HIS3p-neo* reporter gene flanked by *URA3* sequence. First primers MO664 (SEQ ID NO:1) and MO665 (SEQ ID NO:2) were annealed and inserted into the *KpnI-SacI* sites of plasmid pBluescript II KS (Stratagene,). The resulting vector, MB1038, contains a *SalI* site in the polylinker. Next, the *SpeI-XhoI* fragment from pJL164 (Brachmann et al. Yeast 14:115-132 (1998)) containing a deletion of the *URA3* gene with additional flanking sequences was inserted into the *NheI-SalI* sites of MB1038 to create MB1053. Primers MO666

10

15

5 (SEQ ID NO:3) and MO667 (SEQ ID NO:4) that contain multiple restriction sites (*NotI*, *XbaI*, *SpeI*, *XhoI* and *KpnI*) were then annealed together and ligated into the *SmaI* site of MB1053 to create MB1054. Next, the following four fragments were combined in MB1054 to
 10 obtain plasmid MB2254. The *lovF* promoter from *A. terreus* genomic DNA was PCR amplified with MO1794 (SEQ ID NO:5) and MO1793 (SEQ ID NO:6) and inserted into MB1054 on a *NotI*-*SpeI* fragment. The *HIS3* basal promoter from pRS403 (Sikorski and Hieter, *Genetics* 122:19-27 (1989)) was PCR
 15 amplified with primers MO1785 (SEQ ID NO:7) and MO1786 (SEQ ID NO:8) and inserted into MB1054 on a *SpeI*-*BamHI* fragment. Finally, the *neo* gene (PCR amplified with MO195 (*BamHI*) (SEQ ID NO:9) and MO196 (*EcoRI*) (SEQ ID NO:10) from plasmid pYX11 (Xiao and Weaver, *Nucl. Acids Res.* 25:2985-2991 (1997)) and *CYC1* terminator sequences (*XhoI*-*KpnI* fragment from pRS426-GAL-S (Mumberg, et al., *Nucl. Acids. Res.* 22:5767-5768 (1994)) were first
 20 combined in pRS416 (Sikorski and Hieter, *Genetics* 122:19-27 (1989)) and then cut out with *BamHI*-*KpnI* and inserted
 25 into MB1054 to create MB2254.

The *lovFp-HIS3p-neo* reporter in MY2124 can confer resistance to the drug geneticin (G418). It was empirically determined that MY2124 (untransformed or transformed with parental plasmids MB2478 (*CYC1-lovE/CEN*)
 30 or MB2848 (*CYC1-lovE/At274/CEN*) was unable to grow on YPD media supplemented with 100 µg /ml G418. Plasmid MB2478 contains the *CYC1* promoter operationally linked to the entire *A. terreus lovE* open reading frame. The *CYC1* promoter is a relatively weak promoter and thus the *lovE*
 35 ORF in MB2478 was expressed at low levels. MB2478 was the parental vector plasmid for creating full length *lovE* variants. Plasmid MB2848 contains the *CYC1* promoter operationally linked to a chimeric open reading frame

5 consisting of the *A. terreus* *lovE* DNA binding domain
fused to the carboxy-terminal portion of the At274 gene
(U.S. Serial No. 60/257,431, filed December 22, 2000).

MB2848 was used to create *lovE* variants in which the
DNA binding domain was not mutated. Both MB2478 and
10 MB2848 contain yeast CEN and autonomously replicating
sequences and both are maintained at 1-2 copies per cell.
In contrast to strains transformed with MB2478 or MB2848,
strains transformed with plasmid MB1644 (*TEF1-lovE*/2
micron) were able to grow on G418-supplemented YPD media.
15 The *lovE* gene of MB1644 is under control of the
constitutively strong *S. cerevisiae* *TEF1* promoter.
MB1644 contains a 2-micron origin for high-copy
replication in yeast. An objective of these studies was
to identify *lovE* variants which when expressed at low
20 levels could confer G418 resistance similar to the highly
expressed wild-type *lovE* molecule of MB1644. *S.*
cerevisiae expression vectors used in these studies were
constructed as follows.

MB968 is a low copy *S. cerevisiae* *URA3* based
25 expression vector. MB968 was created by inserting the
EcoRV fragment (containing the destination cassette) from
gateway pEZC7201 (Invitrogen™, Carlsbad, CA) into
XhoI/SalI (filled in with Klenow) linearized pRS416 *CYC1*
(Mumberg, et al., *Gene* 156:119-122 (1995)).

30 MB1644 and MB2478 are *URA3*-based *S. cerevisiae*
expression plasmids that contain the wild-type *lovE* gene.
They are both derivatives of MB1199. MB1199 was created
by using primers MO841 (SEQ ID NO:11) and MO842 (SEQ ID
NO:12) to amplify the *lovE* ORF from *A. terreus* cDNA.
35 Gateway (Invitrogen™, Carlsbad, CA) Cloning Technology
(US Patent 5,888,732) was used to clone the *lovE* PCR
fragment into the gateway entry vector pDONR206
(Invitrogen™, Carlsbad, CA) to create MB1199. Similarly,
Gateway Cloning Technology was used to transfer the *lovE*

5 ORF from MB1199 into MB968 to create MB2478 and into
MB969 (U.S. Serial No. 60/198,335, filed April 18, 2000)
to create MB1644.

MB2848 is a derivative of MB968 that contains a
lovE-AT274 chimera. The lovE portion of MB2848 was
10 derived by using oligos MO841 (SEQ ID NO:11) and MO2278
(SEQ ID NO:13) to PCR amplify the lovE DNA binding domain
from *A. terreus* cDNA. A second round of PCR was
performed with primers MO343 (SEQ ID NO:14) and MO2278 to
add appropriate Gateway Cloning Technology compatible
15 sequences. The At274 portion of MB2848 can be derived by
using primers MO2273 (SEQ ID NO:15) and MO2274 (SEQ ID
NO:16) to PCR amplify the carboxy-terminal domain of
At274 from *A. terreus* cDNA. A second round of PCR was
performed with primers MO344 (SEQ ID NO:17) and MO2273 to
20 add appropriate Gateway Cloning Technology compatible
sequences. The lovE and At274 PCR products were cut with
*Bam*HI and purified over a QIAquick PCR purification kit
(Qiagen, Valencia, CA) according to manufacturer's
instructions. Finally, the products were mixed 3-4 hours
25 in a standard ligation reaction and used in Gateway entry
and destination reactions to create MB2848.

Gateway cloning technology was used to clone the
lovE variants of interest into plasmid MB1419 which is a
filamentous fungal expression vector. The MB1419 fungal
30 selection marker is the *A. nidulans* GPD promoter
controlling the *ble* gene from *S. hindustanus*. The
transgene is controlled by the *A. nidulans* PGK promoter.
A. terreus strain MF117 is a derivative of *A. terreus*
strain ATCC 20542.

35

Example 2: PCR Mutagenesis of the lovE DNA Binding Domain

The zinc finger DNA binding domain of lovE is encoded
by nucleotides 100-201 (SEQ ID NO:92). Oligos MO2624

5 (SEQ ID NO:18) and MO2654 (SEQ ID NO:19) were used to PCR
 amplify a *lovE* containing fragment from plasmid MB2478.
 The 1.7 kb product contains nucleotides 212-1410 of *lovE*
 and ~500 bp of flanking vector sequence. Two rounds of
 standard PCR (1.5 mM MgCl₂) were performed with Amplitaq
 10 DNA polymerase (Applied Biosystems, Foster City, Ca)
 according to the manufacturer's instructions.

Plasmid MB2848 was cut with *KpnI*-*Bam*HI to release a 1.1
 kb fragment containing the At274 portion of the *lovE*-
 At274 chimeric open reading frame. The remaining 5.5 kb
 15 vector sequence retains the *lovE* DNA binding domain.

Example 3: PCR Mutagenesis of the *lovE* Open Reading Frame

lovE open reading frame insert was prepared
 according to the following procedure. Oligo pairs MO2680
 20 (SEQ ID NO:20) /MO2686 (SEQ ID NO:21), MO2681 (SEQ ID
 NO:22) /MO2686, and MO2700 (SEQ ID NO:23) /MO2701 (SEQ ID
 NO:24) were used to PCR amplify the entire *lovE* open
 reading frame from plasmid MB2478. The PCR products
 differ in the amount of 5' and 3' vector sequence
 25 flanking the *lovE* open reading frame.

PCR was performed using a GeneMorph PCR mutagenesis
 kit (Stratagene, La Jolla, Ca) according to
 manufacturer's instructions to achieve medium and high
 range mutation frequencies.

30 Plasmid MB2478 was cut with *Asp*718/*Xba*I to release a
 1.7 kb fragment. The remaining 5.0 kb vector sequence
 completely lacks *lovE* ORF sequence.

Example 4: Transformation and Selection for G418R

35 Isolates

All PCR products were purified using a QIAquick PCR
 purification kit (Qiagen) according to manufacturer's
 instructions. All vectors were gel purified using a

5 QIAquick gel extraction kit (Qiagen) according to
manufacturer's instructions.

The mutagenesis strategy of Muhlrad et al. (Yeast
8:79-82 (1992)) was used which involves cotransforming a
mutated PCR product and gapped plasmids into *S.*
10 *cerevisiae*, and then screening for *in vivo* recombinants
having the desired phenotype).

Transformation of *Saccharomyces cerevisiae* was
accomplished by the lithium acetate/single-stranded
carrier DNA/polyethylene glycol (LiAc/ss-DNA/PEG)
15 protocol (Woods R.A. and Gietz R.D. *Methods Mol. Biol.*
177:85-97 (2001)) with a 1:5 molar ratio of vector:insert
DNA to generate >55,000 *in vivo* recombinant transformants
on SC-Ura plates. Transformants were transferred by
replica printing to YPD plates containing 100 µg/ml G418
20 and allowed to grow for 2-4 days at 30°C (Figure 1).

Drug resistant clones were confirmed in secondary
assays including growth on G418 concentrations up to 2000
µg/ml. The plasmid-dependence of the phenotype was
determined by observing the re-appearance of drug
25 sensitivity correlating with loss of the library plasmid.
lovE variant plasmids were recovered from promising
candidates (Hoffman and Winston (1986) *Gene* 57:267).
More than 70 *lovE* variants were identified and
definitively characterized by DNA sequence and/or
30 restriction digestion analysis.

Table 3 summarizes the G418 resistance phenotype and
sequence analysis of 26 of these variants.

Table 3: Variant lovE Mutations

lovE allele	lovFp-neo Mediated G418R	MO oligos used for random PCR mutagenesis	Amino Acid Change 1	Amino Acid Change 2	Amino Acid Change 3	Amino Acid Change 4	Amino Acid Change 5	Amino Acid Change 6	Amino Acid Change 7	Amino Acid Change 8	Amino Acid Change 9	Amino Acid Change 10	Amino Acid Change 11
1	-/+	2624/2654	H253R	S341P									
2	+/-	2624/2654	R121W	S133L	S322G								
3	+++	2624/2654	C73R	A83V	T135I								
4	++	2624/2654	C73R	E177G									
5	++	2624/2654	C73R										
6	+/-	2624/2654	C153Y	E197K	T281A								
7	+	2624/2654	C73R	T256A	N466S								
8	+++	2624/2654	C73R	E141V									
9	++	2624/2654	C73R	E303K									
10	+++	2624/2654	Q41K										
16	+++	2680/2686	Q41K	P16A	G23S	T9M	Q362E						
19	+/-	2700/2701	R21H	S34A	Q80H	A84S	E303D	H374D	A440T	A441V	C445S	P469S	
20	+	2700/2701	F31L	T409I									
21	+++	2700/2701	F31L	M97I	E113D	D146N	P163S	N367I	H458Y				
30	+/-	2681/2686	I43V	Q295L									
31	++	2680/2686	F31L	P101S	C153R	C159S	E162K	R293L	S311N				
32	++	2680/2686	L14I	E18V	G138C	E338G	V361L	P389S	N400S				
33	++	2680/2686	Q41R	S174Y	A402T								
34	++	2680/2686	F31L	T52I	P101Q	P108S	V111I						
36	+/-	2700/2701	D85N	I143F	M232I	T315I	S382Y	M385K					
37	++	2700/2701	T46I	Q62R	K77R	S323C	N367Y	V373I					
38	-/+	2700/2701	Q41R	T294I	P310L	G337D	P389L	A394V	G436S				
39	+	2680/2686	T52N	V111I	T139	V184I	T281A						
40	+++	2680/2686	Q41R	D4E	V87I	D110E	E141K	A189T	N276D	T347R	N367I	Q377R	A425T
41	-/+	2680/2686	D131N	S133L	R312G	A429G							
wild-type	-	N/A	N/A										

5

Table 4 summarizes amino acid substitutions that were isolated multiple times, suggesting that they are particularly important for improving *lovE* variant activity on *lovFp-HIS3p-neo* expression.

10

Table 4: *lovE* Mutations Isolated Multiple Times

Amino Acid Change	Number of Times Isolated in <i>lovE</i> 1-41	<i>lovE</i> variant
F31L	4	20, 21, 31, 34
Q41K	2*	10, 16
Q41R	3*	33, 38, 40
T52I/T52N	1 each	34, 39
C73R	6*	3, 4, 5, 7, 8, 9
P101S/P101Q	1 each	31, 34
V111I	2	34, 39
S133L	2	2, 41
E141V, E141K	1 each	8, 40
C153Y/C153R	1 each	6, 31
T281A	2	6, 39
N367I/N367Y	2/1	21, 40, 37
P389S/P389L	1 each	32, 38

* allele was isolated in additional *lovE* variants that were not fully sequenced

Example 5: Increased *lovF-lacZ* Expression in *S. cerevisiae*

15

In order to quantify the increase in *lovF* expression, β -galactosidase activity was measured in *lovE* variant transformed *S. cerevisiae* strains that also harbored *lovFp-lacZ* reporter derivative plasmids. *lovF-lacZ* reporter derivative plasmids were constructed as follows.

20

Plasmid MB1918 contains the *lovFp-lacZ* reporter gene. It can be derived from pRS424 (Sikorski and Hieter (1989) *Genetics* 122:19-27). First, primers MO107 (SEQ ID NO:25) and MO197 (SEQ ID NO:26) are used to PCR amplify the *lacZ* gene from Yep355 (Myers, et al., *Gene*

25

5 45:299-310 (1986)). This *lacZ*-containing fragment was
 inserted into the *Bam*HI-*Hind*III sites of pRS416 (Sikorski
 and Hieter, *Genetics* 122:19-27 (1989)). This same *lacZ*
 fragment can be cut out of the resulting vector with
 10 *Kpn*I-*Not*I and inserted into the same sites of pRS424 to
 create pRS424-*lacZ*. Primers MO1293 (SEQ ID NO:27) and
 MO1294 (SEQ ID NO:28) are used to PCR amplify a 2.09 kb
 fragment of the *lovF* promoter from *A. terreus* genomic
 DNA. The *lovF* promoter fragment was then cut with *Not*I-
*Bgl*III and inserted into *Not*I-*Bam*HI linearized pRS424-
 15 *lacZ*.

Plasmid MB2114 contains the *lovFp*-*CYC1p*-*lacZ*
 reporter gene. It can be derived from pRS424-*lacZ* (see
 MB1918 plasmid construction). Primers MO1787 (SEQ ID
 NO:29) and MO1788 (SEQ ID NO:30) are used to amplify the
 20 264 bp basal *CYC1* element from pRS415 *CYC1* (Mumberg, et
 al., *Gene* 156:119-122 (1995)). This 264 bp fragment was
 inserted upstream of the pRS424-*lacZ* derivative which has
 been digested with *Spe*I-*Bam*HI. Finally, the *lovF*
 promoter from MB1918 was PCR amplified with MO1793 (SEQ
 25 ID NO:31) and MO1794 (SEQ ID NO:32) and inserted into the
*Not*I-*Spe*I sites to create MB2114.

Yeast strains utilized in this study include strains
 MY2145 and MY2159, which are both derived from the *S.*
cerevisiae sigma 1278b strain background; the genotypes
 30 are both strains are as follows: *MATa ura3Δ0 leu2Δ0*
his3Δ::hisG trp1Δ0::hisG. MY2145 and MY2159 contain the
lovFp-*lacZ* reporter plasmids MB2114 and MB1918,
 respectively.

MY2124 transformed with individual *lovE* variant
 35 plasmids was mated to *S. cerevisiae* strains MY2154 and
 MY2159. Diploids were selected on SC-UraTrp media.
 Multiple diploids from each individual mating were
 assayed for *lovFp*-*lacZ* expression using 96 well format β -

5 galactosidase assays. For β -galactosidase assays, cells were transferred from transformation plates to 96-well microtiter plates containing 200 μ l Z buffer. 12 strains were transferred simultaneously using a 12-channel multi-pipettor to scoop cells from transformation plates.

10 Duplicate samples were prepared for all assays. OD_{600} readings were taken on samples in Z buffer. These values were used to normalize for equal cell number in all assays. After determining OD_{600} , 150 μ l of each sample in Z buffer was transferred onto a Millipore Multiscreen

15 Assay System (Nitrocellulose Immobilon NC), filtered, and then washed by filtering 200 μ l Z buffer. 100 μ l Z buffer with β ME and detergents was then added to each well, as was 20 μ l 4 mg/ml ONPG. Reactions were incubated at 30°C, stopped with 50 μ l 1 M Na_2CO_3 , filtered

20 into a polystyrene 96-well assay plate, and OD_{420} was determined for each assay well. β -galactosidase units were determined using the Miller formula ($O.D. 420 \times 1000$) / ($OD_{600} \times \text{minutes} \times \text{volume in mL}$). Z buffer is made by dissolving the following in 1 L of water (16.1 g $Na_2HPO_4 \cdot 7H_2O$, 5.5g $NaH_2PO_4 \cdot H_2O$, 0.75 g KCl and 0.246 g $MgSO_4 \cdot 7H_2O$).

25 Z buffer with detergents and β ME is made as follows: 9.8 ml Z buffer, 100 μ l 20 mg/ml CTAB, 100 μ l 10 mg/ml sodium deoxycholate, and 69 μ l β ME. Control plasmids utilized in these studies included MB968, MB2478 and MB1644.

30 Results of these studies are presented in Figures 2-5, demonstrating increased transcription-activating properties of the *lovE* variants disclosed herein.

Example 6: Secondary Metabolite Production

5 Transformation of filamentous fungi was performed
according to the following procedure. Protoplasts were
generated by inoculating rich media with spores. Spores
were allowed to germinate for about 20 hrs or until germ
tubes were between 5 and 10 spore lengths. The germlings
10 were centrifuged and washed twice with sterile distilled
water and once with 1 M magnesium sulfate. Germlings
were then resuspended in 1M magnesium sulfate containing
approximately 2 mg/ml of Novozyme. Tubes were then
incubated at 30°C shaking at 80 RPM for about 2 hrs or
15 until most of the hyphae were digested and protoplasts
were abundant. Protoplasts were filtered through one
layer of Miracloth. At least one volume of STC was added
and protoplasts were centrifuged. Protoplasts were
washed twice with STC. Protoplasts then were resuspended
20 in 1ml STC and counted in a hemacytometer. A final
concentration of approximately 5×10^7 protoplasts/ml were
frozen in a 9:1:0.1 solution of STC, SPTC and DMSO in a
Nalgene Cryo cooler at -80°C (cools -1°C/min).

Solutions for transformation were as follows: STC
25 (0.8 M Sorbitol, 25 mM Tris-HCl pH 7.5, 25 mM CaCl_2) and
SPTC (0.8 M Sorbitol, 40% PEG 4000, 25 mM Tris-HCl pH 8,
50 mM CaCl_2). Transformation was accomplished according
to the following protocol. 1-5 μg of DNA comprising a
lovE variant according to the invention in a fungal
30 expression vector was placed in a 50 ml Falcon tube. 100
 μl of previously frozen protoplasts were added to the
DNA, gently mixed, and then incubated on ice for 30 min.
15 μl of SPTC was added, followed by mixing by tapping
and incubation at RT for 15 min. 500 μl SPTC was added
35 and mixed well by tapping and rolling, then incubated at
RT for 15 min. 25 mls of regeneration minimal medium was
added, mixed well and poured on plates containing 25 mls

5 of regeneration minimal medium with 2X the concentration
of selection drug.

Transformation plates were incubated at 26°C for 5-6
days or until colonies started to appear. Regeneration
minimal medium contains trace elements, salts, 25 mM
10 sodium nitrate, 0.8 M Sucrose, and 1% agarose at pH 6.5.
The selection drug that was used successfully with *A.*
terreus is phleomycin, a broad-spectrum glycopeptide
antibiotic. Transformants were picked onto new plates
with a toothpick (if the fungus was sporulating) or with
15 sterile forceps (if the fungus did not sporulate).
Purification plates contained minimal medium (same as
regeneration minimal medium but containing 2 % instead of
0.8 M sucrose) and 1X drug concentration. Picked
transformants were incubated at 26°C for 5-6 days.

20 Transformants were grown in production media for
secondary metabolite production. Briefly, for *A. terreus*
and lovastatin production, spores were used as the
inoculum. Spores were obtained from the purification
plate by using a wooden inoculation stick. The medium
25 was RPM containing corn steep liquor, sodium nitrate,
potassium phosphate, magnesium sulfate, sodium chloride,
P2000 (Dow chemical), trace elements and lactose or
glucose as carbon source. The medium was pH 6.5. Flasks
were incubated at 26°C with shaking at 225 RPM. For
30 static 96-well cultures, the same medium was used and the
spores were obtained from the purification plate with a
wooden toothpick. 96-well plates were incubated, without
shaking at 26°C.

Sampling was done after after 5 days for
35 lovastatin. For shake flask experiments 1-1.5 mls of
supernatant was placed into 96-well plates, which were
centrifuged and supernatants transferred to new 96-well
plates. Samples were frozen at -80°C for storage or for
later assays.

5 Cultures that were grown standing in a 96-well plate were centrifuged and the supernatant was transferred to a new 96 well plate. Samples were frozen at -80°C.

Example 7: Measurement of Secondary Metabolite Production

10 The concentration of the secondary metabolite lovastatin was determined by enzyme inhibition assay (Figure 6). Briefly, 10 µL of sample was removed and diluted 1:100 in H₂O. 10 µl of this diluted broth was assayed in a reaction (200 µL total) containing 1 mM

15 HMGCoA, 1 mM NADPH, 0.005 mM DTT and 5 µl (His)₆HMGR. The disappearance of absorbance at 340 nm was observed over time. This represents the disappearance of NADPH, and lovastatin inhibits this reaction.

The initial velocities were calculated for the

20 reactions containing samples, adjusted for dilution, and compared to reactions containing lovastatin standards to determine levels of metabolite produced. (His)₆HMGR was expressed in *Saccharomyces cerevisiae* and purified with a nickel column.

25 The results from ten individual transformants for each allele are shown in standard box plot format in Figure 6. Lovastatin concentration from the corresponding wild-type *lovE* control is shown in matching fill pattern. For example, *lovE* alleles 2, 7, 8 and 9

30 were all transformed and assayed at the same time as the non-hatched wild-type control. The horizontal line in each individual box represents the median.

Lovastatin concentration was also determined by high pressure liquid chromatography (HPLC). Briefly, 100 µL

35 of broth sample was removed and diluted 1:10 into 70% H₂O-30% acetonitrile (900 µl). This mixture was spun down to pellet debris at 13000 RPM for 5 minutes. 900 µl of this

5 diluted broth was transferred to a vial and the sample
 was analyzed by HPLC. 10 µl were injected into a Waters
 HPLC system (996 photo-diode array detector, 600 E pump
 controller and 717 autosampler) equipped with a YMC-Pack
 ODS column (Aq-302-3, 150 x 4.6 mm ID, S-3 µM pore size)
 10 and eluted with isocratic 40% aqueous acetic acid (0.7%) -
 60% acetonitrile for 8 minutes. Lovastatin was detected
 at 238 nm to have a retention time of 6.5 minutes and was
 quantified using a calibration curve created from pure
 lovastatin samples.

15 The results from ten individual transformants for
 each *lovE* variant are shown in standard box plot format
 in Figure 7A and 7B. Thirty individual wild-type *lovE*
 transformants and ten individual MB2143 negative control
 transformants were tested. Identical controls are
 20 plotted in Figures 7A and 7B.

PCR analysis of *A. terreus* transformants
 demonstrates that greater than fifty percent of the
 transformants contain the transgene. Variability in
 levels of transgene expression can presumably be
 25 influenced by integration site and copy number. *lovE*
 variants containing identical amino acid substitutions
 are labeled.

The amino acid and nucleic acid sequences of *lovE*
 variant sequences are presented in Table 5 and Table 6,
 30 respectively.

Table 5: Amino Acid Sequences of Variants of the *lovE* Gene

<i>lovE</i>-1
maadqgftnsvtlspvegsrtggtlprrafrrrscdrchaqkikctgnkevtgrapcqrcqqagl rcvysercprkrklrqsraadlvsadpdpclhmssppvpssqslpldvsseshssntsrqfldppdsy dswtsigtdeaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq relfddlsavsqeileeillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadcrqgtldec lrtnlftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd tipffsenlpigelfpyvdplthalfsacttlhvgvqllreneitlgvhsaaggiaasismsepg ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark hkhqmlrldlennipp (SEQ ID NO:41)

love-2

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
 rcvysercpkrklrqsraadlvadpdpclhmssppvpqsqslpldvseshssntswqfldppdsy
 dwlwtsigtdeaaidtdcwglscqcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
 relfddlsavsgeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadchqgtldec
 lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghgsvd
 tipffsenlpigelfsyvdpplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
 ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
 hkhgmlrdlnnipp (SEQ ID NO:42)

love-3

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
 rcvyserprkrklrqsrvadlvadpdpclhmssppvpqsqslpldvseshssntsrqfldppdsy
 dwswisigtdeaaidtdcwglscqcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
 relfddlsavsgeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadchqgtldec
 lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
 tipffsenlpigelfsyvdpplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
 ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
 hkhgmlrdlnnipp (SEQ ID NO:43)

love-4

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
 rcvyserprkrklrqsraadlvadpdpclhmssppvpqsqslpldvseshssntsrqfldppdsy
 dswtsigtdeaaidtdcwglscqcdggfscqleptlpdlpspfestvgkaplppvssdiaraasaq
 relfddlsavsgeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadchqgtldec
 lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
 tipffsenlpigelfsyvdpplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
 ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
 hkhgmlrdlnnipp (SEQ ID NO:44)

love-5

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
 rcvyserprkrklrqsraadlvadpdpclhmssppvpqsqslpldvseshssntsrqfldppdsy
 dswtsigtdeaaidtdcwglscqcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
 relfddlsavsgeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadchqgtldec
 lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
 tipffsenlpigelfsyvdpplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
 ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
 hkhgmlrdlnnipp (SEQ ID NO:45)

love-6

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
 rcvysercpkrklrqsraadlvadpdpclhmssppvpqsqslpldvseshssntsrqfldppdsy
 dswtsigtdeaaidtdcwglscqcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
 rklfddlsavsgeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadchqgtldec
 lrtnklftavhcyilnvrilaaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
 tipffsenlpigelfsyvdpplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
 ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
 hkhgmlrdlnnipp (SEQ ID NO:46)

love-7

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
 rcvyserprkrklrqsraadlvadpdpclhmssppvpqsqslpldvseshssntsrqfldppdsy
 dswtsigtdeaaidtdcwglscqcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
 relfddlsavsgeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadchqgaldec
 lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
 tipffsenlpigelfsyvdpplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
 ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
 hkhgmlrdlnsipp (SEQ ID NO:47)

love-8

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvyserprkrklrqsraddlvsadpdpclhmssppvpsqslpldvsseshssntsrqfldppdsy
dswtsigtdeaaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadchqgaldec
lrtnknlftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvqvqllreneitlgvhsaaggiaasismsgpegg
ediartgatnsarceegpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnsipp (SEQ ID NO:48)

love-9

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvyserprkrklrqsraddlvsadpdpclhmssppvpsqslpldvsseshssntsrqfldppdsy
dswtsigtdeaaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadchqgaldec
lrtnknlftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvqvqllreneitlgvhsaaggiaasismsgpegg
ediartgatnsarceegpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnsipp (SEQ ID NO:49)

love-10

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvyserprkrklrqsraddlvsadpdpclhmssppvpsqslpldvsseshssntsrqfldppdsy
dswtsigtdeaaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadchqgaldec
lrtnknlftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvqvqllreneitlgvhsaaggiaasismsgpegg
ediartgatnsarceegpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnsipp (SEQ ID NO:50)

love-16

maadqgiftmnsvtlsavegsrtsgtlprrafrrrscdrchakkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraddlvsadpdpclhmssppvpsqslpldvsseshssntsrqfldppdsy
dswtsigtdeaaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadchqgtldec
lrtnknlftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvqvqllreneitlgvhsaaggiaasismsgpegg
ediartgatnsarceegpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:51)

love-19

maadqgiftnsvtlspvegshtggtlprrafrrracdrchaqkikctgnkevtgrapcqrccqagl
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dswtsigtdeaaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadchqgtldec
lrtnknlftavhcyilnvriltaiselllsqirrtqnshmspldgssrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvqvqllreneitlgvdsaggiaasismsgpegg
ediartgatnsarceegpttpaarvlfmflsdegafqeaksagsrgrtitvlrrsyedifslark
hkhgmlrdlnnips (SEQ ID NO:52)

love-20

maadqgiftnsvtlspvegsrtggtlprralrrscdrchaqkikctgnkevtgrapcqrccqagl
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dswtsigtdeaaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadchqgtldec
lrtnknlftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvqvqllreneitlgvhsaaggiaasismsgpegg
ediartgatnsarceegpitpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:53)

love-21

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dswtsigtdeaiddncwglsgcdggfscqlestlpldlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvqvllreieitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkygmlrdlnnipp (SEQ ID NO:54)

love-30

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrchaqkvkctgnkevtgrapcqrccqagl
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dswtsigtdeaiddncwglsgcdggfscqleptlpldlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtlnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvqvllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:55)

love-31

maadqgiftnsvtlspvegsrtggtlprralrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraddvsadpdpclhmssppvpsqslpldvsshsntsrqfldppdsy
dswtsigtdeaiddncwglsgcdggfssqkptlpldlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvqvllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:56)

love-32

maadqgiftnsvtlspvgsrtggtlprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraddvsadpdpclhmssppvpsqslpldvsshsntsrqfldppdsy
dswtsigtdeaiddncwglsgcdggfscqleptlpldlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvqlllreneitlgvhsaggiaasismsgesg
ediartgatnsarceeqpttpaarvlmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:57)

love-33

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrcharkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraddvsadpdpclhmssppvpsqslpldvsshsntsrqfldppdsy
dswtsigtdeaiddncwglsgcdggfscqleptlpldlpspfeytvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvqvllreneitlgvhsaggiaasismsgepg
ediartgatnstrceeqpttpaarvlmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:58)

love-34

maadqgiftnsvtlspvegsrtggtlprralrrscdrchaqkikctgnkevigrapcqrccqagl
rcvysercpkrklrqsraddvsadpdpclhmssppvpsqslsldiseshsntsrqfldppdsy
dswtsigtdeaiddncwglsgcdggfscqleptlpldlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvqvllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:59)

love-36

maadqgiftnsvtlspvegsrtggtlprrafrsrscdrchaqkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsrailvsadpdpclhmssppvpsqslpldvsseshssntsrqfldppdsy
dswtsigtdeafdtcdwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvvgvqllreneitlgvhsaaggiaayisksgepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:60)

love-37

maadqgiftnsvtlspvegsrtggtlprrafrsrscdrchaqkikcignkevtgrapcqrccqagl
rcvysercpkrklrqsrailvsadpdpclhmssppvpsqslpldvsseshssntsrqfldppdsy
dswtsigtdeaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvvgvqllreyeitlgvhsaaggiaasismsggepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:61)

love-38

maadqgiftnsvtlspvegsrtggtlprrafrsrscdrcharkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsrailvsadpdpclhmssppvpsqslpldvsseshssntsrqfldppdsy
dswtsigtdeaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpidelfsyvdpplthalfsacttlhvvgvqllreneitlgvhsaaggiaasismsgelg
edivrtgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:62)

love-39

maadqgiftnsvtlspvegsrtggtlprrafrsrscdrchaqkikctgnkevngrapcqrccqagl
rcvysercpkrklrqsrailvsadpdpclhmssppvpsqslpldiseshssntsrqfldppdsy
dswtsigideaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppissdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvrilaaaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvvgvqllreneitlgvhsaaggiaasismsggepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:63)

love-40

maaeqgiftnsvtlspvegsrtggtlprrafrsrscdrcharkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsrailvsadpdpclhmssppvpsqslplevsseshssntsrqfldppdsy
dswtsigtdekaiddcwglsgcdggfscqleptlpdlpspfestvekaplppvssditraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyildvriltaiselllsqirrtqnshmsplegsrsqspsrddtssssghssvd
tipffsenlpigelfsyvdpplrhalfsacttlhvvgvqllreieitlgvhsargiaasismsggepg
ediartgatnsarceeqpttpaarvlfmflsdegatfqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:64)

love-41

maadqgiftnsvtlspvegsrtggtlprrafrsrscdrchaqkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsrailvsadpdpclhmssppvpsqslpldvsseshssntsrqfldppdsy
nwlwtsigtdeaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspsgddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvvgvqllreneitlgvhsaaggiaasismsggepg
ediartgatnsarceeqpttpaarvlfmflsdegafqegksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:65)

Table 6: DNA Sequences of Variants of the *lovE* Gene***lovE-1***

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGCACATGTCCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCCCGGCAGTTTCTTGATCCACCGGACAGCTAC
 GACTGGTCTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTGAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAAGTGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCTGCGCCAACAAGCGCAGGCCGACTGCCGTCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACAGCAGCAGCGGCCAGCAGCAGTGTGAC
 ACCATAACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:66)

lovE-2

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGCACATGTCCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCCTGGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTGAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAAGTGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACGGCAGTGTGAC
 ACCATAACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:67)

lovE-3

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTATGCACAAAAGATCA
 AATGTACTGGAATAAGGAGGTTACTGGCCGTGCTCCCTGTGATCGGTGTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGACGCCCCAAGCGCAAGCTACGCCAATCCAGGGTAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGATCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTGAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGTGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCGGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCTCCATGA (SEQ ID NO:68)

lovE-4

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTATGCACAAAAGATCA
 AATGTACTGGAATAAGGAGGTTACTGGCCGTGCTCCCTGTGATCGGTGTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGACGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTGAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGTGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCGGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCTCCATGA (SEQ ID NO:69)

lovE-5

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGACGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACCTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGTGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTTCAATGCGTTCACGAC
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 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTTGAC
 ACCATAACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCCAGGGCATTTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACTACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTCGAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:70)

lovE-6

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATATGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAAAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACCTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGTGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTTCAATGCGTTCACGAC
 GGCTTCTTACTGTCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGCCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTTGAC
 ACCATAACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCCAGGGCATTTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACTACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTCGAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:71)

lovE-7

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTGACGCTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGACGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTGAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACCTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCGCACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATAACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCCAGGGCATTGACGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTCGAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAGTATTCTCCTCATGA (SEQ ID NO:72)

lovE-8

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTGACGCTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGACGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGTGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTGAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACCTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATAACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCCAGGGCATTGACGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTCGAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCTCATGA (SEQ ID NO:73)

lovE-9

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTGACGCTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGACGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 TTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCAC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTGAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACCTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAACGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGAAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATAACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:74)

lovE-10

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCAAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTGACGCTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTGAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACCTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATAACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:75)

lovE-16

ATGGCTGCAGATCAAGGTATATTTCATGAACTCGGTCACTCTCTCTGCAGTGGAGGGTTACGCGAC
 CAGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTATGCAAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTGACGCTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCTCAAATACCTCCCGGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACAGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCACAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAAGTGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTATCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACTAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATAACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTAGAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCCTCCATGA (SEQ ID NO:76)

lovE-19

ATGGCTGCAGATCAAGGTATATTTCACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACACAC
 CCGTGGAACATTACCCCGCCGTGCATTCCGACGCGCTTGTGATCGGTGTATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTGACGCTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCATTCCAGGGCATCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCTCAAATACCTCCCGGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCACAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAAGTGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGACG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATAACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTATTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTAGACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCACAGTACTGCGACGAAGCTATGAGGATATCTTTCCCTCGCCCGCAAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCCTTCATGA (SEQ ID NO:77)

lovE-20

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCACTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTGACGCTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTGAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGCAGGAAGTGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTTGAC
 ACCATAACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCGAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGATCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCCTCCATGA (SEQ ID NO:78)

lovE-21

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCACTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTGACGCTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATATCCTCGCCTCCAGTGCCCTCACAGAGCTTACCGC
 TAGACGTATCCGATTGCGATTCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTAACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTGAGTTAGAGTCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGCAGGAAGTGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCTAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTTGAC
 ACCATAACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGATTGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCGAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCAACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAAA
 CACAAATATGGCATGCTCAGAGACCTCAACAATATTCCTCCATGA (SEQ ID NO:79)

lovE-30

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTATGCACAAAAGGTCA
 AATGTACTGGAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCACTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACCTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTACAGAC
 GGCTTCTTACTGTCTGCGCCAACAAGCGCAGGCGGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCTGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATAACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCGGAGGTCGAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCTCCATGA (SEQ ID NO:80)

lovE-31

ATGGCTGCAGATCAAGGTATATTACGAACTCCGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTACGACGCTCTTGTGATCGGTGTATGCACAAAAGATCA
 AATGTACTGGAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGTTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCCTCGCCTTCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ACGTGATGGAGGCTTCAGCTCTCAGTTAAAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACCTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTACAGAC
 GGCTTCTTACTGTCTGCGCCAACAAGCGCAGGCGGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTACTGTGCGAAATTAGGCTGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAACAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATAACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCGGAGGTCGAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCTCCATGA (SEQ ID NO:81)

lovE-32

ATGGCTGCAGATCAAGGTATATTCACTAACTCGGTCACTATCTCGCCAGTGGTGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTGATCGGTGTCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCCTCGCCTCCAGTGCCCTCACAGAGTTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACCTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGTGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCAACGAC
 GGCTTCTTACTGTCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTTGAC
 ACCATAACCCTTCTTTAGCGAGAACCTCCCTATTGGTGGGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGCTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCCAGGGCATTGCGAGCTTCCATCAGCATGAGCGGGGAATCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTCGAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCTCCATGA (SEQ ID NO:82)

5

lovE-33

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACGAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTGATCGGTGTCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 ATACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACCTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGTGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCAACGAC
 GGCTTCTTACTGTCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTTGAC
 ACCATAACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCCAGGGCATTGCGAGCTTCCATCAGCATGAGCGGGGAACAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTCGAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCTCCATGA (SEQ ID NO:83)

lovE-34

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCGAC
 CGGTGGAACATTACCCCGCCGTGCATTGCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTATTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTATACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCAAGTGCCCTCACAGAGCTTGTCGC
 TAGACATATCCGAGTCGCATTCCTCAAATACCTCCCGGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAAGTGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGTGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCATTCCAGGAGGCAAAGTCTGCTGGTT
 CCGGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCCTCCATGA (SEQ ID NO:84)

lovE-36

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCACCAGTGGAGGGTTACGCGAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGAATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTACACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCTCAAATACCTCCCGGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTTTTGACACTGACTGCTGGGGGCTATCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGCTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAAGTGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGTGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATCTTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAGCAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACATCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTACATCAGCAAGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTGTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCGGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCCTCCATGA (SEQ ID NO:85)

lovE-37

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTATTGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAACGGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAGGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCCCGGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTGAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGGGCCAGTGCACAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAAGTGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCTTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCTCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCGGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCTGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGTATGAGA
 TTACTCTGGGAATACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAAA
 CACAAACATGGCATGCTCAGAGATCTCAACAATATTCCTCCATGA (SEQ ID NO:86)

lovE-38

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACGAAAGATCA
 AATGTACTGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAAGCTGGACTT
 CGATGCGTCTATAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCCCGGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTGAGCTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGGGCCAGTGCACAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAAGTGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTCGCAAATTAGGCGGATCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCTGAGCAGAGACGACACCAGCAGCAGTAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGATGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAAGTAGGC
 GAGGATATAGTCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAAGTGAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCCTCCATGA (SEQ ID NO:87)

lovE-39

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCACCAGTGGAGGGTTACGCGAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTAATGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
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 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCCAGAGCTTGCCGC
 TAGACATATCCGAGTCGCATTCCTCAAATACCTCCCGGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTCGTGGACCTCGATTGGCATTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
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 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGGCCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCGAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:88)

lovE-40

ATGGCTGCAGAACAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCGAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACGAAAGATCA
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 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
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 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
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 GGAGTCGATCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAG
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 TTACTCTGGGAGTACACTCCGCCCCGGGCATTGCGAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:89)

lovE-41

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 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCCTCACCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 AACTGGTTGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAAT
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 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTTCAATGCGTCAACGAC
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 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
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 GGAGTCGATCCCAGTCGCGGAGCGGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATAACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTA CTCTGGGAGTACACTCCGCCCAGGGTATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGGAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCTCATGA (SEQ ID NO:90)

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Equivalents

Those skilled in the art will recognize, or be able
 to ascertain, using no more than routine experimentation,
 10 many equivalents to the specific embodiments of the
 invention described herein. Such equivalents are
 intended to be encompassed by the following claims.

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